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HARD WHITE WHEAT IMPROVEMENT

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"HARD WHITE WHEAT IMPROVEMENT"

FINAL REPORT
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ABSTRACT

The principal objective of this project was to develop selection protocols and germplasm to aid in the development of hard white bread wheat cultivars with quality comparable to Canada Western Red Spring (CWRS) wheat. Many of the white wheat lines developed derive from backcrossing white alleles into CWRS cultivars in an attempt to maintain the quality associated with CWRS wheat. However, as the red pericarp allele is dominant to the white pericarp allele, selections must be made for white versus red grain color. To accomplish this, a non-destructive method of color differentiation to facilitate screening of white versus red grain kernels is essential. As seed availability is a limiting factor in many breeding programs, an efficient means of color sorting while maintaining seed viability is required. A 5% NaOH protocol is often used for identification of red versus white seed, however it results in decreased germination. In order to overcome this, various concentrations and soak times of NaOH were evaluated on a test set of four genotypes of wheat varying in the number of alleles contributing to pericarp color (RL4137 - 3 genomes with red pericarp alleles; Glenlea - 2 genomes with red pericarp alleles; Red Bobs - 1 genome with red pericarp alleles; and W98616 - white line, no red pericarp alleles). Concentrations of NaOH tested ranged from 1% to 25%, and 5% NaOH was identified as the optimum concentration for color differentiation causing minimal seed damage. Color development reached its peak for differentiation after 30 minutes of treatment. Methods comparing the degree of application from soaking the grain in salt solution to a small application by needle pricking were tested. A minimal application of 5% NaOH as applied by pricking the brush end of the kernel with a needle was effective in color differentiation with no significant effect on germination, compared to soaking the kernels in NaOH or KOH. To facilitate color sorting of larger samples of grain, while allowing for seed to remain viable for use in field multiplications, reduced volumes of NaOH and KOH were evaluated. When treating the grain with 1.0 to 1.5 mL of 5% NaOH or KOH the only significant treatment effect was on seedling density while the remainder of growth variables were not affected. Therefore, 1.0 mL NaOH or KOH and 1.5 mL KOH treatments are effective for color differentiation without causing a decrease in germination. The use of a Satake ScanMasterII color sorter was evaluated for seed color separation. When tested on segregating F_2-F_4 bulk populations, seed identified as white and planted the following year consistently saw an increase in the percent of white seeded grain in the following generation. Application of minimal volumes of NaOH to grain prior to color sorting resulted in increased percentages of white grain in the populations.

To date the majority of Fusarium Head Blight (FHB) resistance breeding has been focused on red wheat cultivar development. A second objective of this project was to integrate FHB resistance genes into elite lines of hard white wheat using molecular marker assisted selection facilitated backcrossing. In five of six backcross-derived populations selected for the presence of two major FHB QTL (3BS+5AS) there was evidence of significant improvement in FHB reaction relative to the recurrent parent.

INTRODUCTION

Background Information

The major objective of this project was to develop selection protocols and germplasm to aid in the development of hard white bread wheat cultivars with quality comparable to Canada Western Red Spring (CWRS) wheat. In order to accomplish this objective, a protocol that allows for the non-destructive differentiation of red and white wheat kernels is beneficial. As well, Fusarium Head Blight (FHB) resistance breeding has primarily been targeted in red wheat cultivar development. A second objective of this study was to incorporate FHB resistance genes into white grained wheat using molecular marker assisted backcrossing.

Currently CWRS varieties account for the majority of wheat production in Canada (63.3%; 2009 Canadian Wheat Board Variety Survey). The CWHWS class was established in 2001, with the intention of encouraging wheat production targeted for Asian noodle and bread markets (McCallum and DePauw 2008). Approximately 12% of global wheat production is used for producing noodles (Cato et al. 2006). Australia has traditionally led production of hard white wheat. Australian hard white wheat breeding has focused on the quality expectations of Asian markets, which account for 40% of its export market (Crosbie et al. 1998). Similarly, the Asian market accounts for >50% of Canadian wheat exports (Hatcher et al. 2006). Asian markets tend to prefer hard white wheat for noodles (Lang et al. 1998), lending to the increased potential for hard white wheat production in Canada.

There remains a large potential for growth in Canadian hard white wheat acreage, as in 2008, only 0.6% of total wheat acres were seeded to CWHWS, 99% of which was the variety Snowbird (2008 Canadian Wheat Board Variety Survey). Acreage of CWHWS increased only slightly to 0.8% in 2009 (2009 Canadian Wheat Board Variety Survey). However, a significant change was seen in the percent acreage of CWHWS varieties grown, as the acreage of Snowbird seeded was significantly reduced to 48.8%, and the recently released variety Snowstar accounted for 49.3% (2009 Canadian Wheat Board Variety Survey). Many of the white wheat lines developed derive from backcrossing white seeded alleles into CWRS cultivars, in order to obtain the desired color while maintaining the high quality standards of CWRS (Ambalamaatil et al. 2002).

Breeding for white kernel color as opposed to red has several advantages including brighter flour, increased flour extraction rate, which concurrently leads to increased protein concentration, decreased bitterness, and decreased speckiness in noodle production (Ambalamaatil et al. 2002; Hatcher et al. 2006; Matus-Cádiz et al. 2008). Three major homoeologous loci located on the group three chromosomes of wheat are known to control kernel color (Metzger and Silbaugh 1970; McIntosh et al. 2003). Red is dominant to white color, and the presence of a single red allele is enough to confer red color to the grain (Metzger and Silbaugh, 1970). Expression of the degree of red color is additive (Sherman et al. 2008). In a simple red by white cross, the expected ratio of white to red grain is 1:63.

Current protocols available for the differentiation of grain color often result in reduced seed viability. Color differentiation has commonly been reported using salt solutions of sodium

hydroxide (NaOH) or KOH (Lamkin and Miller 1980; De Pauw and McCaig 1988; Dowell 1997). Concentrations and temperatures of application for the chemical have proven effective for color differentiation, however often with disregard for the negative impacts on seed viability. Therefore a means of separating white from red seed without reducing viability would be beneficial to a breeding program.

Fusarium Head Blight (FHB) continues to be an issue for wheat production. Much of the effort thus far has been to develop red-grained cultivars with resistance. In the long-term, limits on vomitoxin (DON) levels in wheat will be the same for all major wheat classes. Thus, it would be prudent to introduce FHB resistance genes into CWHW wheat from red-grained sources. Molecular markers for Sumai3-derived lines are available (for example: maswheat.ucdavis.edu/protocols/FHB/index.htm. Due to the low probability of combining white seed color with FHB resistance in one F2 genotype from a biparental cross we proposed to develop a series of populations which are based on marker-assisted backcrossing to elite CWHW recurrent parents. Two backcrosses (or equivalent) would be made (*3), giving us 87.5% recovery of the recurrent phenotype. Following the last backcross the resulting lines would be selfed and then grown in a field FHB nursery to assess the effectiveness of MAS. The most promising lines would then be advanced for agronomic and quality evaluations.

METHODS AND RESULTS

METHODS

1. Determination of non-destructive procedures for differentiation of red and white-grained wheat

1.1 NaOH

Previous reports in the literature have suggested the use of sodium hydroxide (NaOH) to facilitate color enhancement for differentiation of red and white grained wheat. This method aids in the objective differentiation of red and white kernels that are often difficult to distinguish visually. NaOH reacts with the grain to turn red pericarps a red-brown in color and white pericarp cream-yellow. However, the suggested volumes, concentrations, and soak times are destructive to the kernel, often rendering the seed non-viable. Therefore, the objective of this study was to determine if it was possible to develop a protocol involving NaOH that facilitates color differentiation without reducing seed viability. Several protocols were developed to test varying methods, rates and times of application. Five different lines were selected to determine differentiation of red/white color with varying number of genomes containing red pericarp alleles including: RL4137 (red pericarp alleles in 3 genomes), Glenlea (red pericarp alleles in 2 genomes), Red Bobs (red pericarp alleles in 1 genome), W98616 (no red pericarp alleles) and ND690 (no red pericarp alleles).

In 2006, field trials were conducted at Kernen Crop Research Farm and the Seed Farm at Saskatoon, Saskatchewan to obtain seed for field testing. Genotypes included in the trials varied in the number of red pericarp alleles present in the three genomes, including RL4137,

Glenlea, Red Bobs, and W98616. Trials were grown in a randomized, complete block design (RCBD) with four replications. Plots measured 1.2 m wide x 3.7 m long, with 5 rows spaced 24 cm apart, and were seeded at a rate of 1400 seeds per plot. Single heads were picked at harvest and threshed in a belt thresher to obtain seed.

1.1.1 NaOH concentrations

Previous reports have recommended use of a 20% NaOH solution for screening (Ram et al. 2002), while 5% NaOH is routinely used in our breeding line verifications (to ensure HWS seed increased is not contaminated with red grain). In order to determine an effective concentration for non-destructive differentiation of red and white kernels, the five different lines identified above varying in the number of genomes with red pericarp alleles were evaluated. Two replications of eight seeds of each line were examined for preliminary analysis, with decreased soak times for increased concentrations of NaOH. Six concentrations of NaOH (0.4 mL for each treatment) were evaluated on the five lines: 1, 5, 10, 15, 20, and 25%. Kernels were soaked in NaOH until optimum color was developed, then rinsed three times with distilled water and placed in petri dishes to germinate for five days.

1.1.2 NaOH Soak Time Optimization

Following determination of an acceptable concentration of NaOH for color differentiation, an experiment was performed to determine the optimal soak time for red/white color differentiation in a 5% NaOH solution without negatively affecting seed viability. Three repetitions of eight seeds were soaked in 0.4mL of 5% NaOH at 20°C in an incubation cabinet. A water control was also included in the experiment. Seeds were soaked in 5% NaOH solution for durations of 15, 30, 45 and 60 minutes. Following the soak time, seeds were rinsed three times with distilled water before being placed in petri dishes with moistened filter papers for germination (5 days at 20°C).

1.1.3 Half seed treatment

As whole kernel treatment with 5% NaOH caused a decrease in germination, wheat kernels were cut in half to determine whether it would be possible to treat the non-embryo end of the seed with 5% NaOH, and germinate the embryo end, without decreasing germination (at 12°C). This method was able to distinguish between red and white genotypes, however it was difficult to differentiate between red genotypes.

1.1.4 NaOH Injection (Pricking) method

To reduce the impact of 5% NaOH on the kernel, application of the solution to a small part of the kernel was investigated. Three different size needles, small, medium (25 gauge), and large (16 gauge), were used to scratch the kernel through the upper layer of the epidermis opposite the end of the embryo in order to apply a drop of 5% NaOH. Four kernels were tested for each of the five lines in the bulk populations, and placed in Petri dishes with moistened filter paper to germinate at 20°C.

To further test the modified method developed for applying 5% NaOH by pricking the brush end of the kernel, seed of the four lines for testing was obtained from 2006 field trials grown at Kernen and Seed Farm, Saskatoon. The effectiveness of three different treatments (5% NaOH seed pricking, 5% NaOH soak, and a water control) were compared for their ability to differentiate color without reducing seed viability. Fifty kernels were placed on a filter paper with 3mL distilled water, and incubated at 20°C overnight for 16 hours to allow seeds to rapidly imbibe water. The following morning, imbibed seeds were pricked with a small needle, allowing a drop of 5% NaOH to be applied to the seed. After color determination, seed was placed at 12°C to germinate for 10 days. For the 5% NaOH soak treatment, 50 kernels of wheat were placed in 5% NaOH for 30 minutes, rinsed three times with water and blotted dry. Seed was then placed in a petri dish on filter paper moistened with 3 mL of distilled water, and placed in 12°C incubator and allowed to germinate for 10 days. The same procedure was also followed using a water control for germination to determine the effect of the NaOH soak and prick application methods on the germination of respective genotypes. All treatments were repeated four times for samples from each plot.

Growth Experiment

All three methods, 5% NaOH soak, seed pricking, and the water control were examined in a growth experiment in order to determine the treatment effects on emergence, plant growth and yield on seed of the genotypes previously described. Three replications of approximately 70 seeds per line were seeded. Pots were seeded at a rate of 25 seeds (pre-germinated) per 15 cm pot in a growth chamber with 23°C -18 hour days, and 18°C - 6 hour nights. Emergence counts were taken every day for 5 days, following which the pots were thinned to 8 plants per pot, and fertilized (20-20-20) at the three leaf stage. Fresh and dry shoot weight were measured on half the pots at the five leaf stage, and the 50% spike emergence and physiological maturity, plant height and yield were recorded on the remaining pots.

Segregating Populations

To further explore the ability of the pricking method to accurately distinguish red from white grain, 28 F_2 or F_3 bulk populations from the CDC Spring Wheat Breeding program segregating for red and white kernel color were evaluated using the three methods. For each population, a selection of 20 kernels, ten red and ten white, were identified using the pricking method without rinsing, then germinated at 12°C and transferred to pots (10 plants per 15 cm pot), from which single plants were harvested at physiological maturity. From each of the single harvested plants, six seeds were examined using the seed pricking method.

1.1.5 KOH treatments

In 2007, a different technique for color differentiation of red and white kernels using potassium hydroxide (KOH) was brought to our attention and was tested following the USDA KOH method (Anonymous, 2003). This method uses a 25% KOH bleach solution, which when mixed with grain for 1.5 to 2 minutes, rinsed and blotted dry, produces similar kernel color changes to NaOH. To test the relative performance of this method to differentiate color versus other solutions, and the effect of bleach in the treatment, the 25% KOH bleach solution, KOH in water solution, 5% NaOH soak, and a water control were compared.

Genotypes tested were RL4137 (red pericarp alleles in three genomes) and W98616 (no red pericarp alleles).

The 25% KOH in bleach treatment was performed on a 15 g sample of grain in a beaker with 10 g KOH pellets and 40 mL of bleach added. The solution was mixed for 2 minutes, rinsed with warm tap water, and blotted dry. From this, 50 kernels were removed to dry overnight. The 25% KOH in water treatment used a solution of 20 g of KOH mixed with 80 mL water. Forty mL of solution was added to each 15 g sample and allowed to soak for 2 minutes, followed by rinsing 3 times with water, and blotting to dry. The NaOH treatment soaked 50 kernels in 5% NaOH for 30 minutes, samples were then rinsed three times with water and blotted dry. Fifty kernels from each of the treatments were placed on filter paper in a petri dish with 3 mL water and allowed to germinate for 10 days at 12°C.

1.1.6 Reduced volume applications of 5% salt solutions for non-destructive color differentiation

Base concentration

In 2008, reduced volumes of 5% NaOH and 5% KOH were tested to determine whether it would be possible to differentiate grain color without reducing seed viability. A set of three different volumes (2.0 mL, 1.5 mL, 1.0 mL) of 5% NaOH and 5% KOH were evaluated on four genotypes varying in their degree of redness (RL4137, Glenlea, Red Bobs, and W98616). The genotypes tested were obtained from field trials grown in Saskatoon in 2006. Each volume of salt solution was applied to fifteen grams of grain from each field plot in a 50 mL centrifuge tube, which was routinely shaken until all of the solution was absorbed. Treated sample color was then measured on all of the samples, as well as on a set of untreated controls using a Hunter Lab colorimeter (models No 45/0-L MiniScan XE, Hunter Associates Lab Inc., Reston, VA, USA). Three measures of color based on light reflectance were obtained from the Hunter Lab – L*, a* and b*. L* value is a measure of brightness, with an L* of 100 being perfectly white, and of 0 being perfectly black. a* measures red to green color, with positive values being redder and negative values being greener. b* measures yellow to blue color, with positive values relating to greater yellow color, and negative values to blue color. Differences in color were calculated between each of the red (RL4137, Glenlea and Red Bobs) and white (W98616) genotypes. From each of the samples, four runs of 50 seeds were germinated in a petri dish on filter paper using 3.0 mL distilled water, and incubated at 12°C for 10 days.

Crop Growth

Three salt treatments (1.0 mL 5% NaOH, 1.0 mL 5% KOH, and 1.5 mL 5% KOH) chosen based on the base concentration experiment, from 2008 were applied to four genotypes (RL4137, Glenlea, Red Bobs, W98616). The salt treatments, along with untreated control seed were planted at a rate of 304 seeds/m^2 in the field at Saskatoon as a four replicate RCBD, in 3.7 m long, five row plots, with 23 cm spacing in 2008 and 2009. Growth factors including days to emergence, seedling density, days to heading, days to maturity, plant height, yield, grain test weight, and grain seed weight were measured. Seedling density was determined based on an average of three quadrat seedling counts per plot from a 0.0929 m^2 area.

1.2 Reflectance Probe

The use of a reflectance probe was investigated for application in color differentiation. Using the visible light spectra, seed was placed longitudinally, crease down, and slopes of reflectance versus wavelength were compared. Comparison of red (RL4137) and white (ND690) genotypes showed different slopes of reflectance versus wavelength using visible light only, however when tested with both visible and UV light ranges, the reflectance probe did not clearly differentiate the genotypes. Using NIR, none of the lines could be separated when the number of kernels being tested was increased. Based on these results, no further experiments were conducted using this method.

1.3 Mechanical color sorting

After the first year of protocol development, the seed pricking method appeared to be effective for color differentiation; however this method is not suitable for sorting large samples processed in breeding programs. A potential solution for sorting large samples is the use of a color sorter. A Satake ScanMaster II color sorting machine was acquired by the Pulse group of the Crop Development Center for use in their breeding program to sort different colored seed samples. The manufacturer attested to the ability of the machine to sort through heterogeneous colored samples of a number of crops, however no reports or protocols were available for using the color sorter to sort wheat. The color sorter determines grain color using cameras which view the front and back of the grain separately against opposing colored backgrounds through selected color filter lenses. Backgrounds are chosen based on the color of the sample and the desired color differentiation outcome. Based on set thresholds, the grain is sorted into either an accept bin by being allowed to continue to flow through, or rejected with a controlled burst of air to the grain if it does not meet the threshold value.

The ScanMaster is not 100% accurate and is prone to making both Type I and Type II errors when sorting heterogeneous samples. Type I error occurs when white grain is sorted to the reject bin, whereas a Type II error is a result of red grain in the accept bin. For the purposes of color sorting for the breeding program, a Type II error is of greater concern. It would be more beneficial to have a smaller sample size, with some white grain being sorted to the reject bin with the red seed (Type I error), than a larger volume (accept) sample with more red contaminants.

1.4 Fusarium Head Blight (FHB) Resistance

Breeding for resistance to Fusarium Head Blight (FHB) has largely been aimed at red grain wheat. One of our objectives is to use molecular markers to partially backcross FHB resistance genes into elite white grain lines.

The molecular breeding portion of this project was initiated in early 2005 with the initial crosses being completed in the spring. Red-grained lines with resistant reactions (Sumai3-based) to FHB were obtained from Dr. Julian Thomas (AAFC-CRC) and used as the donor

parents. A series of white seeded lines from the CDC that were identified during the course of our first HWS wheat project as well as Snowstar (BW315a) from AAFC CRC (Dr. Gavin Humphreys) were used as the recurrent parent. The first backcross was made during August of 2005. The BC1F1 seedlings were germinated at 12C to break seed dormancy and planted in a growth room in October-November of 2005. Three seeding dates, each a week apart, were used in order to stagger the subsequent crossing program and to allow time for the marker determination. Three major QTL for FHB reaction have been identified in the literature (3BS, 5AS and 6B). We were unable to implement the marker protocol for the QTL on 6BS. In any case, we developed populations which potentially could carry the 6B QTL and could eventually be screened downstream in the breeding program. Seedlings in the BC_2F_1 generation were screened with the 3BS and 5AS QTL markers in March of 2006 and the selected seedlings were crossed to three new HWS wheat parents identified during our 2005 agronomic and quality testing cycle.

We grew the resulting F_1 generation in a growth room after genotyping. The resulting F_2 populations from the BC_1 and BC_2 crossing cycles were grown in 1.3 x 1.3m bulk plots at Saskatoon in 2006 using standard small plot techniques. A subset of populations was sent for winter nursery increase (2006/2007) and was grown as F_4 bulks (1.3 x 3.6 m) in 2007. A group of nine populations were advanced two generations via single seed descent in the greenhouse (winter 2006/2007). The nine populations were grown out in F_5 hills in 2007. These hills were inoculated with leaf and stem rust but hot dry conditions in 2007 resulted in minimal infection levels in this particular nursery. The hills were subsequently sampled after selection for plant type. Six populations were subsequently selected and evaluated for FHB QTL profile in early 2008.

The following method is adapted from Matus-Cádiz MA, CJ Pozniak, P Hucl, GR Hughes. 2006. A simplified multiplex PCR method to screen for the major QTL carrying Fusarium head blight resistance in Sumai-3 (Canadian Journal of Plant Science 86: 711-716). This simple, rapid, and inexpensive PCR-based FHB-MAS method was developed to enable breeders to rapidly develop adapted Canadian cultivars which carry at least the major FHB resistance gene on 3BS.

Plant Material

Sumai-3, BW315a, recurrent parental genotypes and 465 F_1 seeds from backcrossed populations were grown under growth chamber conditions. Sumai-3 and BW315a were included as positive and negative controls. Conditions were set to 20/18ºC (day/night) with 18h light and a photosynthetically active radiation level of 250 $\mu mol\ m^{-2}\ s^{-1}$. Pots were filled with Terra-Lite Redi-Earth (W.R. Grace and Co. of Canada Ltd. Ajax, ON) and seeded at a rate of three seeds per pot. Seedlings were watered every 4d and fertilized using Type 100 Nutricote controlled release granular fertilizer (14-14-14) (Plant Products Co. Ltd. Brampton, ON) at a rate of 0.8-kg m^{-2}. Capped centrifuge tubes (2-mL), each containing one 6-mm glass bead (Cat. No. 26396-621; VWR international; Mississauga, ON), were used to collect 4-cm of tissue once seedlings were 2-wk-old.

DNA Extraction

Leaf tissue within uncapped tubes was desiccated for 24-hr using a maxi-dry lyo (Cat No. 003840; Heto Lab Equipment; Allerod, Denmark). Once dry the leaf tissue within capped tubes was pulverized for 5-min at maximum speed using an in-house modified paint shaker apparatus. Total genomic DNA was extracted from of each seedling using a modified CTAB (hexadecyltrimethylammonium bromide) protocol (Procunier, J.D., Jie, X., and K.J. Kasha. 1991. Barley Genetics Newsletter 20:74-75). To the pulverized leaf tissue, 500 uL of hot (65°C) CTAB buffer (2% CTAB w/v, 20 mM EDTA, 1.4M NaCl, 1% PVP, 100 mM Tris, pH8.0) was added. Tubes were vortexed at medium speed for 3-sec to dissolve as much of the pulverized powder as possible. The mixture was placed at 65°C for 60 min; then one volume of chloroform/isoamyl alcohol (24:1) was added. Tubes were vortexed at low speed for 3-sec to mix. After centrifugation (14 000 rpm) for 5-min (Eppendorf centrifuge model 5810R, Brinkmann Instruments Inc., Mississauga, ON), 400 uL of the upper phase was transferred to a 1.5-mL tube. The DNA was precipitated from the supernatant with 2 volumes of cold (store at -20°C) 95% ethanol. Tubes were stored at –20°C for 20-min or overnight if needed. Following centrifugation (14 000 rpm) for 5-min, the DNA pellet was dried overnight under a fume hood and resuspended in 50-uL of TE (lOmM Tris, lmM EDTA, pH 8.0) for 72-hr prior to utilizing. Resuspended gDNA was stored at 4°C for 3 to 4-wk.

PCR Amplification

Four primer sets were used in the PCR screening including, gwm493 (forward primer 5'TTCCCATAACTAAAACCGCG3'; reverse primer 5'GGAACATCATTTCTGGACTTTG3'), gwm533 (forward primer 5'AAGGCGAATCAAACGGAATA3'; reverse primer 5'GTTGCTTTAGGGGAAAAGCC3'), gwm304 (forward primer 5'AGGAAACAGAAATATCGCGG3'; reverse primer 5' AGGACTGTGGGGAATGAATG3'), and gwm293 (forward primer 5'TACTGGTTCACATTGGTGCG3'; reverse primer 5'TCGCCATCACTCGTTCAAG3') (McCartney, C.A., Somers D.J., Fedak, G., Cao, W. 2004. Haplotype diversity at fusarium head blight resistance QTLs in wheat. TAG 109:261-271). Reactions were performed in 96-well microplates (VWR International Cat No. 82006-636; Mississauga, ON) by adding one drop of light mineral oil (BDH Inc., Toronto, ON; Cat. No. K91960) to each well. All reactions were performed on a PTC-100 MJ Research DNA Thermal Cycler with 2.5 µl of gDNA extract amplified in a 25 µl reaction volume. Prior to adding the master PCR mix, centrifuge the microplate at 2755 x g for 1 min to prevent the gDNA template from evaporating from the wells. For PCR reactions, the mixture consisted of 1X PCR buffer, 1.5 *m*M $MgCl_2$, 0.2 µM of forward primer (Invitrogen, Carlsbad, CA), 0.2 µM of reverse primer, 200 µM of each dNTP (Invitrogen; Cat No. 10297-018), and 0.2 U *Taq* DNA polymerase (Invitrogen, Carlsbad, CA; Cat No. 18038-042). For ease, the 2250 uL master PCR mixture was prepared in a 5 mL tube (VWR International Cat No. 16465-262; Mississauga, ON). After 22.5-uL of master PCR mixture was added to each well, the microplate was centrifuged at 2755 x g for 1 min to ensure the PCR mixture settled at the base of the well. The PCR amplification conditions included an initial 3 min denaturing step at 94°C followed by 45 cycles of denaturing (94°C) for 30 sec, primer annealing for 1 min, extension (72°C) for 1 min, and a final extension of 10 min at 72°C. If needed, reactions were held at 10°C overnight. Primer annealing was performed at 52°C for primers gwm533 and gwm493 and 44°C for primers gwm304 and gwm293.

Electrophoresis

For primers gwm 533 and gwm 493, products from the PCR amplification reactions were separated by electrophoresis at 155 V for 1h and 15 min using a 150 mL 1X TBE (45 m*M* Tris-borate, 1 m*M* EDTA), 2 % (w/v) UltraPure agarose gel (Invitrogen; Cat No. 15510-027) containing 2 uL ethidium bromide (10 mg mL^{-1}; EMD Chemicals Inc., Gibbstown, NJ; Cat. No. CAS1239-45-8). Microplates were centrifuged at 2755 x g for 1 min after adding 4 uL of loading buffer (0.25% bromophenol blue, 40% [w/v] sucrose) to each well. Approximately 10 uL (for gwm533) to 15 uL (for gwm493) from each reaction, containing loading buffer, was loaded per lane and visualized with UV light. The remnant portion of the PCR reaction was stored at –20°C in a sealed plastic bag in case a re-run was desired. Agarose gel analysis was conducted using a Sunrise 96 Biometra horizontal gel electrophoresis apparatus (Mandel Scientific Company Inc.; Cat. No. BM11068111) that allowed for the simultaneous evaluation of 104 samples. Approximately 0.5 ug of 1 Kb Plus DNA ladder (Invitrogen; Cat. No. 10787-018), suitable for sizing DNA fragments from 100 bp to 12 Kb, was loaded into the first and last lanes of each of the four combs. Samples within the gels were scored as possessing a resistant or susceptible genotype relative to the controls (Sumai-3 and BW315a) run concurrently within each gel.

For primers gwm304 and gwm293, products from the PCR amplification reactions were separated as described for gwm533 with the following modifications. For primer gwm304, electrophoresis was performed at 155 V for 1 hr and 30 min using a 150 mL 1X TBE, 2.5 % (w/v) UltraPure agarose gel. For gwm293, electrophoresis was performed at 150 V for 4 hr using a 150 mL 1X TBE, 3% (w/v) metaphor agarose gel (Cat. No. 50180; Cambrex; Rockland, Maine).

RESULTS

2. Determination of non-destructive procedures for differentiation of red and white-grained wheat

2.1 NaOH concentrations

As NaOH concentration increased, the duration of soak time required for visible color differences decreased (Table 1). Of the concentrations tested, 1% NaOH produced a faint difference in color between red and white lines, and was determined to be unsuitable for differentiation. Separation was found to be similar for concentrations of 5, 10, and 15% and 20 and 25% NaOH. Kernels were lighter in color for 20 and 25% NaOH, possibly the result of decreased soak time, while concentrations of 5, 10, and 15% NaOH resulted in color separation which allowed easy differentiation of red and white wheat. In terms of germination, only seed treated with 1% NaOH concentration germinated, all other concentrations were lethal to the kernel. However, kernels were soaked in NaOH at 60°C, which may have led to the reduced germination rates. Based on this information, the lowest possible concentration that differentiated color was chosen (5% NaOH) and the treatment temperature reduced to 20°C to prevent heat reducing seed viability.

2.2 NaOH Soak Time Optimization

Red kernels were distinguishable from white kernels using all of the soak times. Only at 30 and 60 minutes was Red Bobs distinguished from RL4137 and Glenlea, however Glenlea and RL4137 were not distinguishable (Table 2). Germination rates were higher for soak times of 15 and 30 minutes, than for 45 and 60 minutes. It was determined that a larger sample size would be needed to distinguish if a significant difference in germination exists between 15 and 30 minutes, so the experiment was repeated with three repetitions on a larger scale for 15 and 30 minute soak times. Germination rates did not vary greatly between 15 and 30 minute treatments when conducted on 150 seeds (Table 3). Two genotypes, RL4137 and W98616, had low germinations in the water control, suggesting residual seed dormancy. Previous work has proven that germinating dormant wheat lines at 12°C is effective for breaking dormancy (Matus-Cadiz and Hucl, 2003), therefore further germinations were conducted at 12°C.

2.3 Half seed treatment

Cutting the kernel showed no apparent decrease in germination (Table 4). Though the method was effective, it would be too laborious due to labelling requirements for large scale screening as needed in a breeding program.

2.4 NaOH Injection (Pricking) method

Pricking with the small needle required the use of a dissecting microscope, and did not clearly distinguish genotypes. The larger size needles provided better differentiation between red and white genotypes, but not clear differences among red genotypes. This method resulted in a slight decrease in germination for all lines, possibly caused by exudates from the scratch on the kernel resulting in an increase of fungal infection to the seed (Table 5). Due to the time consuming nature of the two step process used initially, the pricking method was modified to a one step procedure where seeds were set up for germination to imbibe water, and the following morning (approximately 16 hours later), a small needle with a droplet of 5% NaOH was used to prick the seed, and after standing for 30 minutes, color differentiation was possible under the dissecting microscope. Seed was then germinated at 12°C. The modification of imbibing the seeds prior to treatment prevented exudates from the seed, thereby reducing the opportunity for fungal infection and did not result in a reduction in germination when compared to a water control (Table 6).

Analysis of germination percentages indicated the largest source of variation was due to treatment effects (73%; Table 7). The NaOH soak method had significantly lower germination (78.8%) than either the seed pricking method (99.7%) or the water control (99.1%) (Table 8). There was a significant interaction between treatment and genotype, likely the result of Glenlea and W98616 having lower germination percentages for NaOH then Red Bobs and RL4137 (Table 8). There was also a significant interaction between location and genotype, with Glenlea having the lowest percent germination at the Seed Farm, and W98616 the lowest percent germination at Kernen (Table 9).

Rinsing

For application in breeding programs, it would be more practical to avoid rinsing seed due to resource and time requirements involved. Therefore, four runs were conducted comparing seed that was not rinsed after pricking, to seed that 30 minutes following the pricking procedure was rinsed three times with water to evaluate negative impacts on germination. No significant differences were found among genotypes, however, significant differences were identified among seed treatments (df=1, MS=289.00, F value= 20.78, p=<0.001). Rinsing the seed after pricking resulted in 99.9% of the seed germinating, compared to the slightly lower 95.6% germination of unrinsed seed, though significantly different. However, when germination was measured in the germination velocity experiment, there was no significant difference between rinsed and unrinsed seed. Based on the high germination percentage of unrinsed seed, the extra effort to rinse seed is not justifiable.

Pricking with water

To determine whether the decrease in germination of the pricking method was due to NaOH or the physical needle damage, an experiment was conducted comparing the pricking method with 5% NaOH not rinsed, the pricking method with water not rinsed, and an untreated water control. A significant difference in the effect of seed treatments (df=2, MS=152.79, F value=35.89, p=<0.001), where the untreated water control and the pricking method with water resulted in near total germination (99.6% and 99.8%), where as when NaOH was applied with seed pricking, germination decreased (95.9%; LSD = 1.03). Therefore, the lower germination is likely due to the application of NaOH, not the physical damage to the kernel itself.

Germination velocity

The pricking, 5% NaOH soak and water control treatments were tested and compared to determine whether there was a difference in germination velocity. Germination counts were taken every 12 hours for the first five days, then every 24 hours for five days following. The coefficient of velocity of germination was calculated as described by Kader (2005). No significant effect due to treatment was identified for germination velocity, however there was a significant difference due to genotype and the treatment x genotype interaction (Table 11). Red Bobs had the fastest germination rate (measured as the coefficient of velocity (CV) = 33.29), whereas Glenlea had the slowest (CV = 27.48) (Table 12). The fact that Glenlea has the lowest coefficient of velocity and Red Bobs has the highest likely contributes to the significant effect of the genotype x treatment interaction. The treatments did not significantly affect germination velocity, but did affect final percent germination, with the water control and pricking treatments having significantly higher germination percentages (99.6% and 97.1%; LSD=2.38) then the NaOH treatment (83.4%)

Growth Experiment

No significant difference among genotypes or treatments was found for seedling emergence after five days, with all showing nearly 100% emergence. There was a significant difference among replicates, genotypes and seed treatments for the coefficient of velocity of emergence (Table 13). W98616 was the fastest to emerge (41.10) (not significantly different from Red Bobs; 39.91, LSD = 1.65), and Glenlea the slowest (37.48), though not significantly different

from RL4137 (39.16). The 5% NaOH treatment was significantly faster to emerge (43.31) than either the water control (37.23) or the pricking method (38.71; LSD=1.73), which did not significantly differ from one another. However, the final emergence percentages between treatments were not different. The faster emergence of 5% NaOH was possibly due to the high pH of 5% NaOH (pH = ~12.6) neutralizing the low pH of the soil (pH = ~5.5), creating more favourable conditions.

There were no significant differences for fresh weight and grain yield. Dry weight, heading date, and plant height all showed a significant effect due to genotype (Table 14). Days to maturity showed a significant effect due to both genotype and rep. The significant effect due to rep on days to maturity was likely caused by the outer wall of the growth chamber being cooler than the inner wall, resulting in a decreased rate of growth in that rep compared to the others. Treatment however did not show a significant effect on plant growth, indicating that the pricking and NaOH treatments do not negatively effect growth.

Segregating Populations

The seed pricking method was able to correctly identify 27/28 populations segregating for red and white color. The population in which color was not correctly identified is believed to be the result of a seed mix up during seeding, therefore the pricking method is able to correctly identify grain color.

2.5 KOH treatments

Both 25% KOH solutions in bleach and in water resulted in color changes similar to those caused by the 5% NaOH treatment in genotypes RL4137 and W98616 (Table 15). The bleach in the 25% KOH solution did not appear to positively enhance color differentiation or negatively impact germination when compared to the 25% KOH in water solution. However, the KOH treatments did result in substantially reduced germination when compared to the untreated check, however, it was unclear as to whether the KOH treatments resulted in decreased germination as compared to the 5% NaOH treatment. Comparison of the 25% KOH solution in bleach and water revealed that the bleach solution did not separate red and white kernels better than the water solution. Therefore, further experiments conducted used the KOH water solution.

When the KOH method was compared to other treatment types, the majority of variation was caused by treatment and was highly significant (Table 16). The NaOH treatment had the lowest percent germination of all of the treatments (70.7%), compared to the high germination rates of the pricking and water control treatments, which were not significantly different from one another (99.3% and 99.8%, LSD = 1.15). The germination of KOH treated seed was significantly lower than either the pricking or water control treatment (97.3%), however was considerably higher than the NaOH treatment. There was a significant difference in germination among genotypes. Red Bobs and RL4137 had significantly higher germination (93.2% and 93.1%) than Glenlea and W98616 (90.2% and 90.6%, LSD = 1.15). The interaction between treatment and genotype was highly significant, as a result of diverging germination percentages for NaOH treated seed, with Red Bobs and RL4137 having higher germination than W98616 and Glenlea (Table 17).

After determination of the potential of the KOH method, treatments of 25% KOH in water, 5% NaOH soak, 5% NaOH prick, and the water control were compared using four runs of all four genotypes (RL4137, Glenlea, Red Bobs and W98616). The same protocol was followed as described above, with the exception that 6 mL of 25% KOH solution was applied to 50 kernels of wheat.

Germination Velocity

The germination velocity experiment from 2006 was repeated with two runs using the Seed Farm field trial as the seed source (only one location was used as location was determined not to have an effect on germination velocity) to include the KOH treatment method. The KOH treatment was included in the 2007 field experiment. Between the second and third days, the pricking and control treatments showed rapid germination in comparison to the NaOH and KOH treatments (as indicated by the slopes of the curves for germination, Figure 1). Within the pricking and control treatments, genotype curves showed only slight differences, as compared to the NaOH and KOH treatments, which exhibited greater variation among genotypes. Among the genotypes, Glenlea was the slowest to germinate for the NaOH and KOH treatments. Final germination after ten days was approximately 100% for the pricking and control treatments, as compared to the NaOH and KOH treatments, whose final germination ranged from 40% to 80%. The coefficient of emergence of velocity showed no significant difference in treatment or years (Table 18), but a significant difference among genotypes, with Glenlea having a significantly lower CVG (27.14) than the other genotypes (Red Bobs 31.2, RL4137 29.5, W98616 29.3; LSD=2.05). The interaction between treatment and year was significant, with the pricking treatment showing a larger CVG in the second year. The genotype x treatment interaction was also significant, with Glenlea having a low CVG in the NaOH and KOH treatments. Based on the above results, this study indicated that the speed of germination is a result of differences in genotype, not seed treatment.

Final Germination

In 2007, the germination experiment from 2006 was repeated on remaining grain from 2006 field experiments at Seed Farm and Kernen to include the 5% KOH treatment, and subsequently the 5% KOH treatment was included in the 2007 field trial. The majority of variation in the experiment was a result of treatment effects (approximately 72%) and was significant (Table 19). There was no significant difference between the control and pricking treatments (99.7% and 97.9%), however these treatments were significantly different from the 5% KOH and 5% NaOH treatments (72.2% and 57.9% respectively; LSD=20.6). There was a significant effect due to genotype, the result of Glenlea having a significantly lower germination (76.5%, LSD = 4.6) than the other genotypes which were not significantly different from one another (Red Bobs – 86.0%, RL4137 – 82.8%, W98616 – 81.8%). In terms of interactions, there was a significant effect due to genotype by treatment as a result of Glenlea having lower germinations when treated with NaOH or KOH. The year by treatment interaction was significant due to the NaOH treatment having a significantly lower germination percentage in the second year as compared to the first (year 1=63.7%, year 2=50.8%, LSD=6.09). Year x location x treatment, location x treatment x genotype, and year x treatment x genotype interactions were all significant, however their contributions to total variation are minimal.

2.6 Reduced volume applications of 5% salt solutions for non-destructive color differentiation

There was a significant effect due to genotype on L* values, and a highly significant ($p<0.01$) effect due to replication, treatment, and treatment by genotype interaction (Table 20). Treatment and error variances accounted for 54% and 50% of the total variance respectively. Among the different salt solution treatments, only the 1.5 mL 5% KOH, 2.0 mL 5% KOH, and 2.0 mL 5% NaOH had L* color differences greater than the untreated controls, and would be useful for selection of white versus red grain(Table 21). The other treatments (1.5 mL 5% NaOH, 1.0 mL 5% NaOH, and 1.0mL 5% KOH) did not clearly distinguish grain color, showing lower color differences than among the untreated controls.

Replication, genotype, treatment and genotype by treatment interaction all had a highly significant effect on the a* values. The greatest source of variance was due to treatment, which accounted for 72.6% of total variance (Table 22). All of the salt treatments were effective for sorting white grain from red grain, as all had larger, significant color differences then the untreated checks. However, higher volumes of salt solution did not necessarily result in greater a* differentiation among genotypes of red versus white grain. The 1.5 mL 5% NaOH treatment showed the largest difference in a* and was significantly different from the other treatments (Table 21). The treatments of 1.0 mL 5% NaOH, then 2.0 mL of 5% NaOH and 5% KOH followed in the degree of a* differentiation, and finally 1.0 mL 5% KOH and 1.5 mL 5% KOH showing the least amount of a* color difference, yet maintained greater color differences than the control. As expected, RL4137 (1.58) showed the largest a* color difference, significantly higher than Glenlea (1.24) and Red Bobs (1.01, LSD=0.13) (Table 23). There were a number of non-crossover interactions, resulting in a significant genotype by treatment interaction, though the variation due to genotype by treatment was smaller than the variation resulting from treatment.

b* was significantly affected by rep, genotype, treatment and genotype by treatment interaction, however error accounted for the majority of variance (59%) (Table 24). b* measurements of color difference found all treatments differed significantly from the control. Treatments of 1.0 mL 5% KOH, 1.0 mL 5% NaOH, and 1.5 mL 5% NaOH did not have b* values significantly different from one another, however did differ significantly from the untreated control. Treatment with 2.0 mL 5% KOH had the largest differentiation in b*, which was significantly different from 1.5 mL 5% KOH and 2.0 mL 5% NaOH (Table 21). When treated with volumes of 1.5 mL and 2.0 mL, 5% KOH resulted in larger b* color differences than treatment with 5% NaOH, however there were no significant differences with the 1.0 mL volume treatment. As with a*, the greatest difference in b* color by genotype ranged in rank with the number of red alleles per genome, with RL4137 having the largest color difference (6.55), followed by Glenlea (6.16) and Red Bobs (5.46; LSD=0.34). The significant treatment by genotype interaction for b* results from several interactions. RL4137 has a significantly larger b* color difference for the control than the other genotypes. 5% KOH treatments volumes of 1.0 mL and 1.5 mL resulted in no significant b* differences between genotypes.

Analysis of rates of germination found that replicate, genotype, treatment and treatment by genotype interaction were all highly significant, with treatment accounting for 86% of total variation (Table 25). Increased treatment volumes of 5% NaOH and 5% KOH resulted in decreased germination rates. Further, treatment with 5% NaOH resulted in lower germination than did treatment with 5% KOH for each volume. Treatment with 2.0 mL 5% NaOH had a significant impact on germination, with only 12% germination as compared to the untreated control which had a germination of 93%. Treatments of 1.0 mL 5% KOH, 1.0 mL 5% NaOH, and 1.5 mL 5% KOH all had germination greater than 58%, while the remainder of treatments resulted in unacceptable germination levels (<58%). Glenlea had a lower percent germination when treated with 1.5 mL 5% NaOH compared to other genotypes, resulting in the significant genotype by treatment interaction. Average germination ranged from 47.2% for Glenlea to 58.8% for RL4137.

Based on overall performance of the treatments, the lower volumes of 1.0 mL 5% NaOH, 1.0 mL 5% KOH, and 1.5 mL 5% KOH were deemed to be acceptable to apply to color differentiation in a breeding program, as they did not result in germination rates below 50% and significant differences between treatments and controls were evident for a* and b* measurements. a* appears to be the most effective measure for determining color differences, as the majority of variation is due to treatment effects.

Crop Growth

In 2008, seeding took place on May 29. There was no effect of treatment on emergence, as all field plots emerged on the same day. The only growth variable significantly impacted by treatment was seedling density (Table 26). The untreated control had the highest seedling density (21.8 seedlings per 0.0929 m^2). Treatments with 1.0 mL 5% NaOH or 1.0 mL 5% KOH had lower seedling densities not significantly different from one another (19.9 and 19.7, respectively). Treatment with 1.5 mL 5% KOH resulted in the lowest seedling density (17.4; LSD=1.17). There was a significant genotype effect on all of the traits measured. Glenlea had a significantly lower seedling density when compared to the other genotypes (17.5 seedlings per 0.0929 m^2 Table 27).

The crop growth experiment from 2008 was repeated in 2009 at the Seed Farm, Saskatoon, following the same design and protocol. The field trial was planted on June 5, 2009 and the majority of plots emerged on June 14, 2009, with the exception of three plots that emerged on June 16, 2009, however there was no effect due to treatment or genotype on seedling emergence. As in 2008, the only factor significantly affected by seed treatment was seedling density ($p<0.001$) (Table 28). The mean seedling density of the untreated samples (29.8) was significantly higher than the treatments with 1.0 mL 5% NaOH (24.2) and 1.5 mL 5% KOH (24.2; LSD=2.62) (Table 30). Replication had a significant effect on all crop growth factors observed (Table 28). This is not surprising; particularly as in 2009 a low spot in the field was present in the second and third replication. These two replications had a higher seedling density than the outer two replications (Table 31). Remaining factors including days to heading, days to maturity, yield, seed weight, test weight and height were all significantly affected by genotype and replication (Table 28). In all cases, the genotypic effect was of greater significance than the effect due to replication. The only significant effect due to

genotype by treatment interaction occurred for seed weight; however the effect due to genotype was significantly higher than either the effect due to replication or the interaction between genotype and treatment.

Multi-year agronomic data for the four genotypes are summarized in Tables 29, 33 and 36. Data for individual field reps are summarized in Tables 21 and 34 while year effects are summarized in Table 35. A significant interaction between treatment and year was detected for seedling density, however the effect was minimal compared to the large and highly significant effect of year (Table 32).

2.7 Mechanical color sorting

Preliminary experiments were conducted to determine the appropriate color of background and filter lens for red/white grain color differentiation. In order to determine the appropriate combination of colored filter lenses and backgrounds to use, a sample of approximately 5 kg of Snowbird (white pericarp) and Superb (red pericarp) wheat were mixed in equal parts. Using this sample, 30 combinations of the six different filter lenses and five different backgrounds were tested for their ability to recognize and differentiate between white and red wheat kernels. Samples in the final reject and accept bins were tested with 5% NaOH to determine the percent of red and white grain in each of the bins, with the goal of identifying the combination with lowest type II error (least red grain in the accept bin). For the purpose of sorting white and red colored wheat, the optimal background color and lens combination for differentiation was determined to be cream #13 and the XZI filter lens (5% red seed identified in the accept bin versus next lowest combination of marigold #1 and XZI with 8% red seed present in the accept bin) (Table 37).

Though the goal of using the ScanMaster was to sort larger samples generated in breeding programs for color differentiation, the ScanMaster is designed for sorting larger sample sizes, such as generated in seed cleaning operations, whereas breeding programs routinely screen relatively smaller sample sizes. Initial experiments focused on sample sizes of approximately 5 kg, to determine whether wheat could be accurately sorted. To determine whether the ScanMaster was able to sort smaller sample sizes such as those commonly generated in a breeding program, a 1 kg sample was prepared at a ratio of 1 white : 63 red consisting of 16 g of Snowbird (white) and 984 g of Superb (red). Four runs per 'accept' were run through the color sorter. At the end of each 'accept', the grain was set aside, and remaining grain in the 'reject' was resorted as in the first accept. This was repeated for a total of 5 accepts, at which point, samples from each of the 1st to 5th accept bins, and the final reject bin were taken and tested with 5% NaOH for 30 minutes to determine the percent of red versus white grain in each sample (Figure 2). The segregating sample was separated after several runs, with the greatest return after the initial sort. There was a high correlation between the percent of white grain after sorting with both the percent of white grain before sorting and the L* after sorting. There was no correlation between the white grain before sorting and the presence of piebald seed, indicating that presence of piebald in the sample will not decrease sorting efficiency. For the purposes of the breeding program, it was determined that running only first and second 'accepts' are the most practical approach, as the majority of white wheat return is

found in the accept bin after two runs (Table 38). Beyond this point, running additional color sorts to obtain seed requires a considerable amount of time and is not practical for the return.

The color sorter was then applied to sort 106 F_2 and F_3 populations generated from single and three-way crosses that were grown in the field in 2006 after harvest (Table 39). Prior to seeding in 2006, 47 out of 106 populations were color sorted. These populations were sorted a second time following harvest. Color sorted seed was grown in the field, and after harvest, re-color sorting the sample resulted in nearly 100% white grain. The percent of white grain in each population was measured before and after color sorting using a Hunter Lab colorimeter, as well piebald was visually rated prior to sorting. The F_3 generation which had undergone a color sort once previously had a higher percentage of white grain in the starting sample then the F_2 samples which were not previously sorted. The color sorter was able to effectively select white grain from the segregating bulk populations, resulting in a 3.3x increase in white grain in the sorted samples as compared to unsorted. Correlations between piebald and percent white colored grain were not significant, suggesting that the presence of piebald seed does not affect the ability of the color sorter to accurately identify white grain from a segregating population.

2007:

During the year, 98 segregating F_2 populations were color sorted, and an additional 86 F_3 bulk populations were identified to be sorted prior to seeding in 2008 (Table 40). A selection of the 86 F_3 bulks was tested for FHB QTL. Before and after color sorting, subsamples were tested to determine red and white color using the 5% NaOH soak. Forty-seven populations were grown in the field and sorted two years in a row (Table 41). The populations were grown as bulks in 2007 and single spikes harvested (approximately 500) instead of bulk harvesting the populations. Single kernels from each head were tested with NaOH to determine red and white color. Spikes identified as red were discarded and those that were identified as being white were designated to be grown in the field as hills in 2008, with the expectation that the hills should be homozygous white. Color sorting the 98 F_2 bulk populations which were not previously color sorted, found that populations which ranged in 0 to 87% white (average 23%) before sorting, after sorting ranged from 4 to 100% white (average 57%). The relative increase of white kernels ranged from 1 to 17, with an average increase of 4X the white kernels. These increases corresponded with populations relative percent of white kernels before color sorting, where populations which were high in the percentage of white kernels prior to sorting showed the lowest relative increases, and resulted in those populations being 100% white grained. The 47 bulk populations which had been color sorted the two previous years had a very high percentage of white kernels (range 21 to 100% white; average 64%). Similarly, the percent of white single spikes was high, ranging from 26 to 100% (average 67%). Selection for white grained spikes at this point should ensure that the next generation is 100% white and color sorting should no longer be necessary.

2008:

In 2008, 171 F_3 bulk populations from 2007 field plots were color sorted, 69 of which had been previously color sorted (Table 42). These populations were sub-sampled before and

after color sorting, and the percentage of white grain in each sample was measured with 5% NaOH. All populations sorted had an increase in the percent of white grain after sorting. Prior to color sorting, populations ranged from 2% to 100%, and increased to 12 to 100% after sorting. The average percentage of white grain increased from 71% to 90% after color sorting. Populations with 100% white grain were color sorted to remove potential red-grained contaminants. F_2 to F_4 bulk populations from 2008 were color sorted in late 2008, and planted in 2009.

2009:

Following the methods developed in previous years, the Satake ScanMaster II color sorter was used to sort red and white grain from bulk segregating F_2 to F_4 populations harvested in the fall of 2008. 152 segregating populations were color sorted for seeding as bulk populations in spring 2009. In early 2009, 14 segregating F_2 to F_4 bulk plots were selected to color sort using two different methods. The first sort was performed on a dry sample of grain using the color sorter (in the spring of 2009). The second color sort was performed on a sample of grain treated with 5% NaOH solution to determine the effectiveness of using salt treatment for color differentiation to further aid in white versus red grain separation. An initial attempt to sort a 'wet' sample of grain was performed using water. As the color sorter is not designed for sorting wet or damp samples, a minimal amount of solution for color differentiation is desired in order for the seed to absorb the maximum amount of solution. Minimal solution volumes were chosen according to prior base concentration experiments performed for color enhancement. As the color sorter is not designed for wet samples, a minimal volume of solution added to the sample is necessary in order to ensure flow through the gravity slide. Based on the base concentration experiment conducted for the field color enhancement trial, a volume of 1.0 mL distilled water per 15 g seed was tested on 500 g of seed to test seed 'stickiness' and flow in the Satake ScanMaster II. This volume was chosen as it was the smallest volume identified to be effective for color differentiation. Following addition of water, seed was shaken intermittently and allowed to stand for two hours to facilitate water absorption. Three trials with three individual trips were performed through the color sorter, and a very minimal amount of seed 'sticking' was found. Color sorting then proceeded using 1.0 mL 5% NaOH solution per 15 g of seed. The seed and solution were routinely shaken to ensure uniform coverage and absorption, and allowed to stand for two hours to ensure absorption. Two individual bulk plots were seeded from the original F_2-F_4 segregating population bulk plots in 2009 at Kernen Farm, Saskatoon. One plot consisted of dry color sort seed and the second of seed sorted with 5% NaOH solution. It should be noted that the seed sorted using 5% NaOH solution was the remaining seed which had previously been color sorted in its entirety for the dry sample, therefore the ratio of white to red grain present in the sample was already skewed towards red seed, as much of the white grain had been removed from the sample during dry color sorting. In the fall of 2009, 500 spikes per plot were picked from both the dry and 5% NaOH color sorted bulk plots. Spikes were individually threshed using a belt thresher.

To determine the effectiveness of using the addition of 5% NaOH solution to enhance color sorting, seed from each color sorted plot was treated with 5% NaOH for color confirmation. Ten seeds each from 100 spikes harvested were selected from each of the segregating bulk plot populations for testing with 5% NaOH. Following NaOH screening protocol, 10 seeds

were placed in individual petri dishes, and 5% NaOH was added to each petri dish, using a spatula to ensure even coverage of seeds with NaOH. Treated seed was allowed to stand for 30 minutes to facilitate color development. Two controls were included from the 2009 color enhancement field experiment based on pericarp color, W98616 (white pericarp) and Red Bobs (red pericarp, with red alleles present in 1 genome). Each head was scored as producing seed with either white or red color (Table 43). Because the sample from which the NaOH treated material was derived from had been color sorted previous to sorting with NaOH, to confirm efficiency, 500 seeds were counted from the seed source and treated with NaOH following the same protocol for NaOH color screening as described above, including the same check lines. Following color development, the number of red and white seeds were recorded. The percent of white grain following dry sort was 62.7% on average, ranging from 31% to 86%. When examining the plots sorted using 5% NaOH prior to seeding, on average 28.6% of heads are white, ranging from 15 to 45% white. Comparison of the relative percentages of white seed sorted with 5% NaOH and planted in the field in 2009, with percent of white seed in the 2008 reserve seed, (seed source for sort with 5% NaOH) indicate an average increase of five times the amount of white seed after color sorting. All plots showed an increase in white seed after sorting, ranging anywhere from 1.9x to 8.3x more white seed.

2.8 Fusarium Head Blight (FHB) Resistance

Microsatellite markers gwm493 and gwm533 can be used to indirectly select for a major quantitative trait locus (QTL) on chromosome 3BS carrying fusarium head blight (FHB) resistance, in populations derived from 'Sumai-3' derivatives while gwm304 and gwm293 are linked to a FHB QTL on chromosome 5AS. One of the major objectives of this project was to use microsatellite markers which flank either side of QTLs on chromosomes 3BS and 5AS to backcross FHB resistance into advanced hard white wheat lines and enhance FHB resistance in that market class.

Sumai-3 (resistant FHB reaction) and BW315a (susceptible FHB reaction) were included as positive and negative controls. Amplification products observed using gwm533, gwm493, gwm304, and gwm293 in Sumai-3, BW315a, and F_1 seed from backcrossed populations were separated on ethidium stained agarose gels (Fig. 3). For Sumai-3 and BW315a, respectively, amplicon sizes were 160bp and 133bp for locus *Xgwm533-3BS*, 213bp and 157bp for *Xgwm493-3BS*, 233bp and 217bp for *Xgwm304-5AS*, and 218bp and 208bp for *Xgwm293-5AS*. Sumai-3 possesses two amplicons, 160bp (*Xgwm533.1* locus) and 100 bp (*Xgwm533.2* locus), using primer gwm533 (Anderson JA, Stack RW, Liu S, et al. 2001. DNA markers for Fusarium head blight resistance QTLs in two wheat populations (TAG 102 (8): 1164-1168).

For F_1 seedlings expected to be carrying only the 3BS QTL, an initial screen was done with primer gwm533 and only heterozygous lines (carrying the resistant and susceptible allele at the *Xgwn533-3BS* locus) were advanced for screening with gwm493 for heterozygosity at the *Xgwn493-3BS* locus. For F_1 seedlings expected to be carrying only the 5AS QTL, initial screening was performed with gwm304 and only heterozygous lines at the *Xgwn304-5AS* locus were advanced for screening with gwm293 for heterozygosity at *Xgwn293-5AS*. For F_1 seedlings expected to be carrying the 3BS and 5AS QTLs, primers were screened in the following ordered gwm533, gwm493, gwm304, and lastly gwm293. Only F1 seedlings determined to be heterozygous at the *Xgwm533-3BS* locus and then subsequently the

Xgwm493-3BS locus were advanced for screening for heterozygosity at the *Xgwm304-5AS* and *Xgwm-293-5AS* loci. Screening with gwm293 was performed on the least number of lines possible as separation of PCR products involved running metaphor gels for 4-hr instead of agarose gels for 1.5-hr. Only heterozygous F1 plants were used as pollen sources for the next round of backcrossing to recurrent HWS wheat parents.

The parental marker profiles are presented in Table 44. As desired, the recurrent HW parents did not exhibit any of the four markers while the red grained donor parents carried the desired markers for one or both of the desired QTL. The frequency at which the four markers were carried in the BC1F1 is also summarized in Table 44. Of the 465 seedlings assayed, 45% carried one or more of the markers. Roughly 4% carried all four. In crossing to develop the BC_2 population emphasis was placed on using the 4% as the pollen donors. Nearly 800 BC_2F_1 seedlings resulting from crosses with the 4% potential 3BS-5AS carriers were planted for assaying in March, 2006. Thus in little over a year we were able to develop breeding populations which genetically were 87.5% HWS parentage while carrying the major FHB QTL.

The disposal of the populations genotyped in 2006 for the 3BS and 5AS FHB QTL is presented in Table 45. The F_1 plant marker genotype is summarized in the following order: 3BS-5AS-6BS. The available 6BS FHB markers (early 2006) were not used as they were not tightly linked to the QTL. A genotype of ++++nn indicates that the F_1 plant carried the 3BS and 5AS markers but was not expected to carry the 6BS QTL (based on the male parental genotype). A genotype of ++++?? indicates that the male parent did carry 6BS markers but we did not genotype the progeny. Thus a genotype containing more +'s is desirable. A subset of populations was sent for winter nursery increase (06/07NZ destination) and was grown as F_4 bulks in 2007.

Another group of 10 populations was advanced two generations via single seed descent in the greenhouse (AGGH SSD). A total of nearly 5,200 Single Seed Descent lines from six backcross-derived populations in the F5 generation were sown in 2007 and approximately 1,400 of those lines were harvested (Table 46). Culling was on the basis of plant type as there was no leaf or stem rust development in the inoculated nursery that year. The harvested lines were screened for the presence of the 3BS and 5AS QTL in the CDC Wheat Molecular Lab in early 2008 (Dr. Curtis Pozniak). The parental lines for the six populations were tested for the 6BS FHB QTL and none carried the QTL. Thus, the F5 lines were screened for the 3BS and 5AS QTL only. Of the 1,463 lines, 160 carried both QTL. These lines were grown in the Carman FHB nursery as well as in a hill plot at Saskatoon in 2008. The average FHB incidences for the six populations, parental lines and check cultivars are presented in Table 47. One population, X05.615, only had three lines selected out of 170 and these were susceptible to FHB. The three lines are likely a result of cross-overs or QTL mis-identification. For the five remaining populations, the mean of the selected lines was statistically lower (basis a t-test) relative to the recurrent parent(s) for FHB VRI. In the case of three populations (X05.578, X05.607, X05.644) the VRI mean of the QTL selected lines was not significantly different from that of the QTL donor parent. In one population (X05.617) the population mean was significantly higher than that of the donor parent, Alsen. In the case of X05.655 the mean of the population was significantly higher than that one of the donors and

lower than the other (Table 47). Lines which had FHB VRI values of less than 18% (this was less than the mean of BW315a, the most FHB tolerant recurrent white-seeded recurrent parent) were retained and had their seed treated with NaOH in order to confirm their grain color. For two populations (X05.617, X05.655), all the NaOH-evaluated lines were red grained while the remaining populations were mostly white grained (Table 47). Based on the FHB data and grain color determinations, 89 lines were selected for further testing in 2009.

In 2009 FHB levels were lower than in 2008 (Table 48). For the five populations retested in 2009, the FHB VRI mean of the QTL-stacked lines was 2 to 9 times lower than that of the recurrent white-grained parent or BW315a (Table 48). The levels of statistical significance were lower in 2009, likely as a result of less differentiation between resistant and susceptible genotypes as well as smaller sample sizes. However, the pattern observed in 2008 (Table 47) was once again seen in 2009 (Table 48).

CONCLUSIONS AND RECOMMENDATIONS

The 5% NaOH concentration previously employed by the breeding program continues to be the optimum concentration with which to identify red versus white grain, while volume of solution application appears to be a factor contributing to decreases in seed viability. In comparison to the 5% NaOH and 5% KOH soak methods, the pricking treatment only subjects a small area of the grain kernel to NaOH. The pricking treatment was found to be non-destructive to the grain, not significantly affecting germination rates or plant growth, and therefore is the preferred method for color differentiation. Soaking the grain in 5% NaOH and KOH methods proved to be too destructive, rendering the grain wet, soft and unviable. However, when the soaking method was tested at lower volumes (1.0 – 2.0 mL per 15 g grain), color segregation was maintained, and neither germination rates nor plant growth were significantly inhibited. Use of the color sorter in early generations continued to show success for selection of white wheat kernels. Increases in the percent of white seed were consistently seen in F_2-F_4 bulk populations segregating for grain color after each year of sorting. Application of 5% NaOH solution at a rate of 1.0 mL per 15 g seed facilitated mechanical separation of red and white seed and may be used for color sorting to increase selection efficiency. Future studies could be conducted to confirm the effectiveness of using the 5% NaOH treatment in conjunction with a color sorter as compared to color sorting the dry seed.

In five of six backcross-derived populations selected for the presence of two major FHB QTL (3BS+5AS) there was evidence of significant improvement in FHB reaction relative to the recurrent parent. The FHB VRI means for the five populations were 1.5 to 4 times lower than their recurrent parents in 2008 and further skewed in 2009, 2 to 9 times lower after culling of some FHB-susceptible lines. Thus, incorporation of two major FHB QTL appeared to be sufficient to significantly reduce FHB levels. The resulting lines will provide useful parental germplasm for further breeding efforts in the CWHW class. Quality targets have shifted since this project was initiated. The experimental lines we have developed are not likely to meet the new targets. Stacking of additional FHB QTL in hard white wheat backgrounds would be beneficial.

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Table 1. NaOH concentrations and their corresponding soak times for experiment.

NaOH Concentration (%)	Soak Time (min)
1	45
5	40
10	35
15	30
20	27
25	25

Table 2. Effect of NaOH solution soak times on wheat seed germination

Wheat Line	**15 Min**		**30 Min**		**45 min**		**60 min**	
	NaOH Rep 1-3	**Water**	**NaOH Rep 1-3**	**Water**	**NaOH Rep 1-3**	**Water**	**NaOH Rep 1-3**	**Water**
RL4137	2/24 (13%)	5/8	8/24 (38%)	7/8	3/24 (25%)	4/8	1/24 (8%)	4/8
Glenlea	6/24 (33%)	6/8	7/24 (29%)	8/8	1/24 (5%)	7/8	4/24 (22%)	6/8
Red Bobs	8/24 (38%)	7/8	11/24 (45%)	8/8	4/24 (17%)	8/8	4/24 (19%)	7/8
W98616	0/24 (0%)	5/8	1/24 (8%)	4/8	2/24 (33%)	2/8	0/24 (0%)	1/8
ND690	10/24 (42%)	8/8	13/24 (54%)	8/8	5/24 (24%)	7/8	1/24	0/8
TOTAL	**26/120**	**31/40**	**40/120**	**35/40**	**15/120**	**28/40**	**10/120**	**18/40**

Brackets indicate the percentage germination in relation to the untreated check.

Table 3. Germination of the five wheat genotypes after being soaked for 15 and 30 minutes in either 5% NaOH solution or distilled water.

Wheat Line	15 Min		30 Min	
	NaOH Rep 1-3	Water	NaOH Rep 1-3	Water
RL4137	41/150 (57%)	72/150	54/150 (69%)	78/150
Glenlea	32/150 (25%)	128/150	43/150 (31%)	138/150
Red Bobs	71/150 (48%)	149/150	52/150 (35%)	150/150
W98616	66/150 (314%)	21/150	25/150 (47%)	53/150
ND690	62/150 (42%)	148/150	53/150 (38%)	138/150

Brackets indicate the percentage germination in relation to the untreated check.

Table 4. Germination after the kernels had been cut in half and soaked in 5% NaOH compared to the un-cut control treated with 5% NaOH. Brackets indicate the percentage germination in relation to the untreated check.

Line	1st NaOH Rep	2nd NaOH Rep	Total NaOH	Whole Kernel Check
RL4137	13/13	12/13	25/26 (96%)	22/24
Glenlea	13/13	12/13	25/26 (96%)	22/24
Red Bobs	12/13	13/13	25/26 (96%)	24/24
W98616	12/13	10/13	22/26 (85%)	20/24
ND690	13/13	13/13	26/26 (100%)	24/24

Table 5. Germination of wheat seed after being scratched with small needle + 5%NaOH, scratched with small needle + H20, or not treated. Brackets indicate the percentage germination.

Line	Scratch with 5% NaOH			Scratch with H20			No scratch		
	Run 1	Run 2	Total	Run 1	Run 2	Total	Run 1	Run 2	Total
RL4137	7/20	7/20	14/40 (35%)	11/20	11/20	22/40 (55%)	7/20	9/20	16/40
Glenlea	15/20	19/20	34/40 (85%)	17/20	18/20	35/40 (88%)	19/20	17/20	36/40
Red Bobs	17/20	18/20	35/40 (88%)	20/20	18/20	38/40 (95%)	20/20	18/20	38/40
W98616	3/20	2/20	5/40 (13%)	7/20	5/20	12/40 (30%)	12/20	4/20	16/40
ND690	18/20	19/20	37/40 (93%)	18/20	16/20	34/40 (85%)	19/20	19/20	38/40

Table 6. Germination of the five wheat genotypes after being germinated overnight and then pricked with small needle + 5% NaOH or water.

Line	5% NaOH			Water		
	Run 1	Run 2	Total	Run 1	Run 2	Total
RL4137	20/20	20/20	40/40	20/20	20/20	40/40
Glenlea	19/20	20/20	39/40	17/20	20/20	37/40
Red Bobs	18/20	20/20	38/40	20/20	20/20	40/40
W98616	20/20	19/20	39/40	19/20	18/20	37/40
ND690	19/20	20/20	39/40	20/20	20/20	40/40

Table 7. Analysis of variance for final percent germination of four genotypes grown at two different field locations, Kernen and Seed Farm, Saskatoon, in 2006 treated with three seed treatments.

Source of Variation	df	SS	MS	F value	P
Location	1	38.8	38.8	0.11	0.750
Replicate(Location)	6	1302.7	217.1	7.44	<0.001
Genotype	3	228.6	76.2	0.58	0.668
Treatment	2	35993.3	17996.7	268.57	0.004
Location x Treatment	2	134.0	67.0	2.30	0.102
Location x Genotype	3	394.8	131.6	4.51	0.004
Treatment x Genotype	6	708.1	118.0	4.05	0.001
Error	360	10501.6	29.2		
Total	383	49301.9			

Table 8. The interaction of genotype treated with seed treatment for final percent germination from field trials grown at Kernen and Seed Farm, Saskatoon, in 2006.

Treatment	Genotype	Germination (%)
Untreated, Water	Glenlea	99.6
Untreated, Water	Red Bobs	99.3
Untreated, Water	RL4137	98.0
Untreated, Water	W98616	99.4
NaOH	Glenlea	75.3
NaOH	Red Bobs	82.1
NaOH	RL4137	80.4
NaOH	W98616	77.6
Pricking	Glenlea	99.7
Pricking	Red Bobs	99.4
Pricking	RL4137	99.8
Pricking	W98616	99.8
LSD		2.66

Table 9. The interaction of genotype with location for final germination percentage for final percent germination from field trials grown at Kernen and Seed Farm, Saskatoon, in 2006.

Location	Genotype	Germination (%)
Kernen	Glenlea	93.3
Kernen	Red Bobs	93.8
Kernen	RL4137	93.0
Kernen	W98161	91.2
Seed Farm	Glenlea	89.7
Seed Farm	Red Bobs	93.5
Seed Farm	RL4137	92.4
Seed Farm	W98161	93.3
LSD		2.17

Table 10. Analysis of variance for final percent germination of four genotypes treated with two different treatments, the pricking method rinsed or the pricking method not rinsed.

Source of Variation	df	SS	MS	F value	P
Replicate	1	0.0	0.00	0.00	1.000
Genotype	3	9.5	3.17	0.23	0.877
Treatment	1	289.0	289.00	20.78	<0.001
Treatment*Genotype	3	12.5	4.17	0.30	0.826
Error	55	765.0	13.91		
Total	63	1076.0			

Table 11. Analysis of variance for coefficient of velocity of germination for four genotypes treated with three different treatments, an untreated water control, the pricking treatment, or the NaOH treatment.

Source of Variation	df	SS	MS	F value	P
Replicate	3	24.91	8.30	1.22	0.309
Genotype	3	428.58	142.86	20.92	<0.001
Treatment	2	31.09	15.55	2.28	0.109
Treatment*Genotype	6	222.82	37.14	5.44	<0.001
Error	81	553.02	6.83		
Total	95	1260.42			

Table 12. The interaction of four genotypes treated with three different treatments, two of the treatments used to distinguish red from white wheat and one of the treatments represented a control, for coefficient of velocity of germination (CVG).

Gentoype	Treatment	CVG
Glenlea	Untreated water	28.49
Glenlea	NaOH	23.91
Glenlea	Pricking	30.03
Red Bobs	Untreated water	32.29
Red Bobs	NaOH	35.11
Red Bobs	Pricking	32.47
RL4137	Untreated water	28.75
RL4137	NaOH	31.32
RL4137	Pricking	30.29
W98616	Untreated water	31.29
W98616	NaOH	30.22
W98616	Pricking	32.72
LSD		2.60

Table 13. Analysis of variance of coefficient of velocity for emergence for four genotypes treated with three different treatments, an untreated water control, the pricking treatment, or the NaOH treatment.

Source of Variation	df	SS	MS	F value	P
Replicate	2	194.17	97.08	23.19	<0.001
Genotype	3	62.35	20.78	4.96	0.009
Treatment	2	164.26	82.13	19.62	<0.001
Treatment*Genotype	6	60.70	10.12	2.42	0.060
Error	22	92.10	4.18		
Total	35	573.58			

Table 14. Analysis of variance (Mean Squares) of fresh shoot weight, dry shoot weight, heading date, plant height, days to maturity and grain yield for four genotypes treated with three different treatments, an untreated water control, the pricking treatment, or the NaOH treatment.

Source	df	Fresh Shoot Weight	Dry Shoot Weight	Heading Date	Plant Height	Days to Maturity	Grain Yield
Genotype	3	18.00	0.673 *	55.19 **	96.99 *	108.18 **	0.62
Rep	2	23.36	0.066	2.08	87.25	23.08 *	1.14
Treatment	2	1.03	0.056	3.08	53.08	13.58	0.58
Genotype*Treatment	6	9.14	0.136	1.94	23.05	2.62	0.53
Error	22	17.42	0.213	1.84	30.13	4.69	0.76

*,** Significant at the 0.05 and 0.001 levels of probability.

Table 15. Percent germination of red grained RL4137 and white grained W98616 after being treated with four different color treatments and an untreated control.

Treatment	% Germinated	
	RL4137	W98616
25% KOH in bleach	41	39
25% KOH in bleach overnight	64	44
25% KOH in water	52	46
5% NaOH	44	42
Untreated	92	96

Table 16. Analysis of variance for final percent germination of four genotypes treated with four seed treatments (25% KOH in water, 5% NaOH soak, 5% NaOH pricking, untreated control).

Source of Variation	df	SS	MS	F value	P
Replicate	3	80.7	26.9	1.74	0.160
Genotype	3	491.3	163.8	10.59	<0.001
Treatment	3	38263.3	12754.4	824.48	<0.001
Treatment x Genotype	9	1244.3	138.3	8.94	<0.001
Error	237	3666.3	15.5		
Total	255	43745.9			

Table 17. The interaction of genotype with seed treatment.

Genotype	Treatment	Germination (%)
Glenlea	KOH	94.9
Glenlea	NaOH	66.3
Glenlea	Pricking	99.5
Glenlea	Untreated, Water	100.0
Red Bobs	KOH	98.8
Red Bobs	NaOH	75.5
Red Bobs	Pricking	98.9
Red Bobs	Untreated, Water	99.6
RL4137	KOH	97.9
RL4137	NaOH	75.8
RL4137	Pricking	99.1
RL4137	Untreated, Water	99.6
W98616	KOH	97.5
W98616	NaOH	65.1
W98616	Pricking	99.9
W98616	Untreated, Water	100.0
LSD		2.30

Table 18. Analysis of variance for the coefficient of velocity of germination (CVG) four genotypes treated with four seed treatments over two years.

Source of Variation	df	SS	MS	F value	P
Rep (Year)	6	97.6	16.3	3.9	0.001
Treatment	3	3318.0	1106.0	7.2	0.070
Year	1	61.8	61.8	0.4	0.584
Genotype	3	518.6	172.9	13.0	0.032
Genotype*Treatment	9	297.5	33.1	3.8	0.031
Genotype*Year	3	40.0	13.3	1.5	0.277
Treatment*Year	3	464.1	154.7	17.6	<0.001
Genotype*Treatment*Year	9	79.3	8.8	2.1	0.032
Error	218	921.9	4.2		
Total	255	5798.9			

Table 19. Analysis of variance for final percent germination of four genotypes grown at two locations for two years and treated with four seed treatments.

Source of Variation	df	SS	MS	F value	P
Rep(Year Location)	12	1,521.6	126.8	1.89	0.032
Year	1	11,731.6	11,731.6	2.51	0.226
Location	1	685.8	685.8	-----	------
Treatment	3	327,307.6	109,102.5	22.71	0.019
Genotype	3	11,835.4	3,945.1	6.49	0.048
Year*Treatment	3	15,036.1	5,012.0	7.84	0.015
Year*Location	1	2.4	2.4	0	0.947
Year*Genotype	3	1,192.1	397.4	1.17	0.376
Location*Treatment	3	590.8	196.9	0.37	0.781
Location*Genotype	3	941.3	313.8	1.34	0.338
Treatment*Genotype	9	14,315.9	1,590.7	3.66	0.017
Year*Location*Treatment	3	1,213.0	404.3	6.02	<0.001
Year*Location*Genotype	3	309.3	103.1	1.53	0.204
Location*Treatment*Genotype	9	1,791.7	199.1	2.96	0.002
Year*Treatment*Genotype	9	2721.0	302.3	4.5	<0.001
Error	957	64,310.5	67.2		
Total	1023	455,506.1			

Table 20. Analysis of variance of L* color differences for wheat grain treated with six salt treatments or left untreated. The L* color difference values are a measure of the difference between the red genotypes (RL4137, Glenlea, Red Bobs) and the white genotype (W98616).

Source of Variation	df	SS	MS	F value
Replicate	3	74.97	24.99	28.95**
Genotype	2	5.74	2.87	3.32*
Treatment	6	145.60	24.27	28.11**
Treatment x Genotype	12	42.97	3.58	4.15**
Error	312	269.33	0.86	
Total	335	538.62		

*, ** Significant at the 0.05 and 0.001 level of probability, respectively.

Table 21. Average L*, a*, and b* color difference values of grain treated with six salt treatments or left untreated. The color difference values are a measure of the difference between the red genotypes (RL4137, Glenlea, Red Bobs) and the white genotype (W98616).

Treatment	L* Color Difference	a* Color Difference	b* Color Difference
2.0 ml of 5% NaOH	5.56	1.48	6.64
2.0 ml of 5% KOH	6.18	1.39	7.19
1.5 ml of 5% NaOH	4.49	2.14	5.62
1.5 ml of 5% KOH	5.42	1.30	6.53
1.0 ml of 5% NaOH	4.11	1.98	5.52
1.0 ml of 5% KOH	4.76	0.97	5.85
Untreated	4.78	-0.32	5.07
LSD	0.31	0.13	0.43

Table 22. Analysis of variance of a* color differences for wheat grain treated with six different salt treatments or left untreated. The a* color difference values are a measure of the difference between the red genotypes (RL4137, Glenlea, Red Bobs) and the white genotype (W98616).

Source of Variation	df	SS	MS	F value
Replicate	3	5.43	1.81	13.19**
Genotype	2	18.50	9.25	67.34**
Treatment	6	189.59	31.60	230.05**
Treatment x Genotype	12	4.93	0.41	2.99**
Error	312	42.86	0.14	
Total	335	261.32		

Table 23. The L*, a*, and b* color difference interactions of three gentoypes and seven treatments. The color difference values are a measure of the difference between the red genotypes (RL4137, Glenlea, Red Bobs) and the white genotype (W98616).

Genotype	Treatment	L* Color Difference	a* Color Difference	b* Color Difference
Glenlea	2.0 ml 5% NaOH	5.98	1.31	6.99
	2.0 ml 5% KOH	6.55	1.36	7.53
	1.5ml 5% NaOH	4.90	2.00	5.90
	1.5ml 5% KOH	5.67	1.41	6.67
	1.0 ml 5% NaOH	4.50	2.05	5.78
	1.0 ml 5% KOH	5.05	0.96	6.03
	untreated	3.94	-0.38	4.22
Red Bobs	2.0 ml 5% NaOH	4.81	1.07	5.30
	2.0 ml 5% KOH	5.85	1.23	6.73
	1.5ml 5% NaOH	4.35	1.82	5.01
	1.5ml 5% KOH	5.49	1.14	6.43
	1.0 ml 5% NaOH	3.96	1.64	4.98
	1.0 ml 5% KOH	4.75	0.78	5.61
	untreated	5.36	-0.62	4.20
RL4137	2.0 ml 5% NaOH	5.90	2.05	7.63
	2.0 ml 5% KOH	6.15	1.58	7.31
	1.5ml 5% NaOH	4.21	2.62	5.94
	1.5ml 5% KOH	5.09	1.36	6.49
	1.0 ml 5% NaOH	3.88	2.24	5.79
	1.0 ml 5% KOH	4.47	1.19	5.91
	untreated	5.04	0.03	6.81
LSD Value		0.54	0.22	0.75

Table 24. Analysis of variance of b* color difference for wheat genotypes treated with six different salt treatments or left untreated. The b* color difference values are a measure of the difference between the red genotypes (RL4137, Glenlea, Red Bobs) and the white genotype (W98616).

Source of Variation	df	SS	MS	F value
Replicate	3	47.33	15.78	9.61**
Genotype	2	68.17	34.09	20.77**
Treatment	6	160.46	26.74	16.30**
Treatment x Genotype	12	73.48	6.12	3.73**
Error	312	511.99	1.64	
Total	335	861.44		

** Significant at the 0.001 level of probability.

Table 25. Analysis of variance for final percent germination of four genotypes treated with six salt treatments or left untreated.

Source of Variation	df	SS	MS	F value
Replicate	3	1412.9	471	6.72**
Genotype	3	9862.0	3287.3	46.88**
Treatment	6	278884.2	46480.7	662.83**
Treatment x Genotype	18	3686.7	204.8	2.92**
Error	417	29242.1	70.1	
Total	447	323088.0		

Table 26. Analysis of variance (Mean Squares) for crop growth indicators for four genotypes treated with three salt treatments or left untreated.

Source	D F	Seedling Density[1]	Days to Heading	Days to Maturity	Yield kg/ha	Seed Weight g	Test Weight kg/hL	DF	Height (cm)
Rep	3	3.39	0.18	3.68*	613726**	23.50**	2.02**	1	7.03
Genotype	3	34.53**	136.43**	130.35*	9808474**	192.71**	8.22**	3	108.5**
Treatment	3	52.78**	0.14	0.22	9417	0.59	0.04	3	1.2
G*T	9	5.95	0.31	0.77	40182	0.98	0.17	9	10.2
Error	45	3.90	0.27	1.06	35856	2.02	0.27	15	15.0
Total	63							31	

*, ** Significant at the 0.05 and 0.001 level of probability, respectively.
[1]Number of seedlings per quadrat.

Table 27. Average crop growth variables for four genotypes grown at Seed Farm, Saskatoon in 2008.

Genotype	Seedling Density[1]	Days to Heading	Days to Maturity	Height (cm)	Yield (kg/ha)	Seed Weight (g/1000 grains)	Test Weight (kg/hL)
RL4137	20.1	50.1	82.7	105.4	3385	35.7	80.9
Glenlea	17.5	52.8	85.5	111.5	3965	43.2	79.4
Red Bobs	20.1	48.0	81.3	109.6	2156	36.5	79.4
W98616	20.9	54.6	87.7	114.1	2747	36.7	80.1
LSD	1.17	0.31	0.61	3.38	112.5	0.85	0.31

[1]Number of seedlings per quadrat.

Table 28. Analysis of variance (Mean Squares) for crop growth for four genotypes treated with three salt treatments or left untreated.

Source	DF	Seedling Density[1]	Days to Heading	Days to Maturity	Yield kg/ha	Seed Weight g	Test Weight kg/hL	Height (cm)
Genotype	3	11.06	159.38**	124.93**	4086462**	521.15**	9.46**	349.22**
Treatment	3	118.17**	0.79	0.77	36410	1.50	0.15	33.14
Rep	3	43.91*	11.17**	102.35**	1503341**	5.03**	7.50**	242.22**
Genotype*Treatment	9	9.11	0.69	0.34	8591	1.94*	0.05	3.50
Error	45	12.20	0.61	0.82	25615	0.81	0.18	16.07
Total	63							

* Significant at $p \leq 0.05$; ** Significant at $p \leq 0.001$
[1]Number of seedlings per quadrat.

Table 29. Average crop growth performance variable for four genotypes grown at the Seed Farm, Saskatoon in 2009.

Genotype	Seedling Density[1]	Days to Heading	Days to Maturity	Height (cm)	Yield (kg/ha)	Seed Weight (g/100 grains)	Test Weight (kg/hL)
Glenlea	25.2	53.2	91.1	102.0	3717	44.7	77.1
Red Bobs	26.9	48.5	87.7	101.6	3068	34.0	75.6
RL4137	26.9	50.9	88.9	96.1	3240	32.2	76.8
W98616	26.6	55.9	94.1	107.5	2493	34.3	77.4
LSD	2.62	0.59	0.68	3.00	120	0.68	0.32

[1]Number of seedlings per quadrat.

Table 30. Average seedling density per treatment as measured on four genotypes grown at the Seed Farm, Saskatoon in 2009.

Treatment	N	Seedling Density[1]
1.5 mL 5% KOH	16	24.2
1.0 mL 5% NaOH	16	24.2
1.0 mL 5% KOH	16	27.4
Untreated	16	29.8
LSD		2.62

[1]Number of seedlings per quadrat.

Table 31. Average crop growth performance variables for four replications grown at Seed Farm, Saskatoon in 2008.

Replication	Seedling Density[1]	Days to Heading	Days to Maturity	Height (cm)	Yield (kg/ha)	Seed Weight (g/100 grains)	Test Weight (kg/hL)
1	25.8	53.2	94.2	107.6	3586	37.0	77.7
2	27.6	51.8	89.8	100.0	2932	35.8	76.7
3	27.9	51.2	88.8	100.0	3010	35.9	76.1
4	24.3	52.1	89.1	99.6	2989	36.4	76.4
LSD	2.62	0.59	0.68	3.00	120	0.68	0.32

[1]Number of seedlings per quadrat.

Table 32. Combined analysis of variance of crop growth variables measured on four genotypes subjected to three individual salt or control treatments at Saskatoon in 2008 and 2009.

Source	DF	Days to Heading	Days to Maturity	Seed Density	Yield (kg/ha)	Test Weight	Seed Weight (g)	DF	Height (cm)
Genotype	3	293.82***	254.25***	40.54	11334364	12.21	673.63*	3	255.39**
Rep (Year)	6	5.67***	53.02***	23.65**	1058549***	4.76***	14.26***	1	293.27***
Treatment	3	0.57	0.77	149.62	24915	0.03	1.76	3	3.43
Year	1	14.45	1212.78**	1446.41***	139646	322.23***	95.7	1	643.89
Genotype*Year	3	1.99	1.03	5.06	2560527***	5.47***	40.23***	3	7.6
Treatment*Year	3	0.36	0.22	21.33*	20913	0.15	0.33	3	5.89
Genotype*Treatment	9	0.35	0.41	10.43	24062	0.12	2.05	9	9.92
Genotype*Treatment*Year	9	0.65	0.69	4.63	24712	0.1	0.87	9	10.6
Error	90	0.44	0.94	8.05	30736	0.23	1.42	31	21.3

Significant at *0.05, **0.01,***0.001

Table 33. Average crop growth performance variable for four genotypes grown at Seed Farm, Saskatoon in 2008 and 2009.

Genotype	Seedling Density[1]	Days to Heading	Days to Maturity	Height (cm)	Yield (kg/ha)	Seed Weight (g/100 grains)	Test Weight (kg/hL)
Glenlea	21.4	52.9	88.3	107.5	38417	43.9	78.2
Red Bobs	23.5	48.3	84.5	106.8	2612	35.3	77.5
RL4137	23.5	50.5	85.8	101.9	3313	33.9	78.9
W98616	23.8	55.3	90.9	111.7	2620	35.5	78.7
LSD	1.39	0.32	0.47	3.46	86	0.58	0.23

[1]Number of seedlings per quadrat.

Table 34. Average crop growth performance variables for four replications grown at Seed Farm, Saskatoon in 2008 and 2009.

Replication	Seedling Density[1]	Days to Heading	Days to Maturity	Height (cm)[2]	Yield (kg/ha)	Seed Weight (g/100 grains)	Test Weight (kg/hL)
1	22.7	52.3	89.5	109.1	3461	38.1	79.0
2	23.3	51.7	84.5	104.8	2930	36.2	78.1
3	24.0	51.3	85.8		3042	36.6	77.9
4	22.1	51.7	90.9		2952	37.7	78.2
LSD	1.39	0.32	0.47	2.26	86	0.58	0.23

[1]Number of seedlings per quadrat.

[2]Height measurements were recorded only on first two replications of field trial.

Table 35. Average crop growth performance variables by year for field trials grown at Seed Farm, Saskatoon in 2008 and 2009.

Year	Seedling Density[1]	Days to Maturity	Test Weight (kg/hL)
2008	19.7	84.3	79.9
2009	26.4	90.5	76.7
LSD	0.98	0.34	0.17

Table 36. Crop growth as summarized based on interactions between variety and year from field trials grown at Seed Farm, Saskatoon in 2008 and 2009.

Variety	Year	N	Yield	Test Weight	Seed Weight
Glenlea	2008	16	3965	79.4	43.2
Glenlea	2009	16	3717	77.1	44.7
Red Bobs	2008	16	2156	79.4	36.5
Red Bobs	2009	16	3068	75.6	34.0
RL4137	2008	16	33858	80.9	35.7
RL4137	2009	16	3240	76.8	32.1
W98616	2008	16	2747	80.1	36.7
W98616	2009	16	2493	77.4	34.3
LSD			131	0.36	0.89

Table 37. Color sorter lens type and background combinations used in selection of white versus red grain.

Background	Filter Lens	Accept		Reject	
		% Red	% White	% Red	% White
Black #6	FSI	-	-	-	-
Black #6	SVM	-	-	-	-
Black #6	VZI	-	-	-	-
Black #6	XEI	-	-	-	-
Black #6	XEM	-	-	-	-
Black #6	XZI	-	-	-	-
Blue #20	FSI	-	-	-	-
Blue #20	SVM	44	56	41	59
Blue #20	VZI	24	76	71	29
Blue #20	XEI	33	67	51	49
Blue #20	XEM	33	67	52	48
Blue #20	XZI	23	77	84	16
Cream #13	FSI	47	53	38	62
Cream #13	SVM	51	49	38	62
Cream #13	VZI	55	45	44	56
Cream #13	XEI	14	86	78	22
Cream #13	XEM	17	83	72	28
Cream #13	XZI	5	95	70	30
Marigold #1	FSI	-	-	-	-
Marigold #1	SVM	-	-	-	-
Marigold #1	VZI	26	74	79	21
Marigold #1	XEI	-	-	-	-
Marigold #1	XEM	28	72	77	23
Marigold #1	XZI	8	92	67	33
White #15	FSI	-	-	-	-
White #15	SVM	45	55	79	21
White #15	VZI	-	-	-	-
White #15	XEI	29	71	71	29
White #15	XEM	27	73	74	26
White #15	XZI	50	50	87	13

* - indicates that the Satake ScanMaster II could not sense any differences in the heterogeneous sample.

Table 38. Recovery of white seed from a 1 kg sample of a population segregating in a 1:63 ratio of white grain : red grain.

	Weight g (%)	**White g (%)**	**Red g (%)**
1st Accept	47 (4.8)	6 (13)	41 (87)
2nd Accept	24 (2.5)	4 (16)	20 (84)
3rd Accept	25 (2.5)	1 (4)	14 (56)
4th Accept	18 (1.8)	<1 (4)	17 (96)
5th Accept	14 (1.5)	<1 (5)	13 (96)
Total Accept	128 (13.1)	12 (9)	116 (91)
Total Reject	851 (86.9)	2 (0.2)	849 (99.8)

Table 39. Fall 2006 Harvested bulk populations of white x red parental wheat crosses

Plot #	Pedigree	Generation	Previous Color Sorting (CS)	Initial Weight (g)	% White Before CS	% White After CS	Relative increase (X's)	Color After CS			Piebalds Before CS[1]
								L*	a*	b*	
85	BW315a/00H4*J3	F2	none	-	27.4	80.0	2.9	51.26	9.65	27.87	2
86	BW315a/00H4*J3	F2	none	-	26.8	79.8	3.0	52.09	9.59	28.20	2
104	BW315a/BW844	F2	none	-	3.2	7.0	2.2	48.15	10.43	25.69	3
105	BW315a/BW844	F2	none	-	0.4	3.6	9.0	47.66	10.56	25.46	3
106	BW315a/BW845	F2	none	-	4.4	25.0	5.7	49.72	9.46	25.84	3
107	BW315a/BW845	F2	none	-	4.2	25.6	6.1	50.69	9.78	26.85	2
108	BW315a/Harvest	F2	none	-	2.4	12.6	5.3	48.58	10.26	25.09	2
109	BW315a/Harvest	F2	none	-	1.0	6.6	6.6	48.05	10.19	24.73	2
110	BW315a/PT211	F2	none	-	2.0	4.2	2.1	48.74	10.39	25.18	3
111	BW315a/BW297	F2	none	-	8.4	35.6	4.2	48.15	10.24	25.54	4
112	BW315a/BW297	F2	none	-	17.6	39.4	2.2	50.56	9.77	27.01	4
113	BW315a/Alsen	F2	none	-	1.2	6.6	5.5	50.76	11.18	28.51	3
114	BW315a/Alsen	F2	none	-	1.0	3.8	3.8	48.84	10.92	26.45	3
115	BW315a/BW781	F2	none	-	4.8	9.2	1.9	50.43	10.70	27.83	3
116	BW315a/BW781	F2	none	-	1.6	3.0	1.9	50.78	11.04	28.42	3
117	HW02061/00H4*J3	F2	none	-	11.0	82.0	7.5	52.69	9.86	29.28	3
118	HW02061/00H4*J3	F2	none	-	28.0	80.4	2.9	52.23	9.66	28.50	2
119	HW02061/00H4*J3	F2	none	-	18.0	81.4	4.5	53.35	9.89	29.23	2
129	HW02061/BW844	F2	none	-	1.8	4.8	2.7	50.74	10.93	26.70	2
130	HW02061/BW844	F2	none	-	3.6	12.2	3.4	51.12	10.24	27.38	2
131	HW02061/BW844	F2	none	-	2.0	6.0	3.0	49.64	11.05	26.94	2
132	HW02061/BW845	F2	none	-	6.0	24.2	4.0	51.43	9.80	26.29	3
133	HW02061/BW845	F2	none	-	5.4	16.4	3.0	50.57	10.16	26.36	3
134	HW02061/BW845	F2	none	-	6.4	21.6	3.4	48.78	9.82	24.38	2
135	HW02061/Harvest	F2	none	-	1.2	3.6	3.0	47.72	10.05	24.19	2
136	HW02061/Harvest	F2	none	-	1.0	3.6	3.6	49.53	10.27	25.76	2
137	HW02061/Harvest	F2	none	-	1.6	5.2	3.3	51.54	10.42	27.66	2
138	HW02061/PT211	F2	none	-	1.4	4.0	2.9	52.28	9.56	25.09	2

139	HW02061/PT211	F2	none	-	1.8	6.0	3.3	51.95	10.08	25.63	2
140	HW02061/PT211	F2	none	-	1.4	4.0	2.9	51.83	9.72	24.85	3
141	HW02061/BW297	F2	none	-	3.0	29.4	9.8	49.80	9.90	25.23	4
142	HW02061/BW297	F2	none	-	5.4	25.4	4.7	50.45	10.03	25.53	4
143	HW02061/BW297	F2	none	-	6.2	32.6	5.3	50.86	9.60	25.71	4
144	HW02061/Alsen	F2	none	-	1.4	7.0	5.0	50.82	9.70	25.07	4
145	HW02061/Alsen	F2	none	-	2.0	6.4	3.2	50.04	10.77	27.08	2
146	HW02061/Alsen	F2	none	-	1.0	4.8	4.8	51.71	10.70	27.18	2
147	HW02061/BW781	F2	none	-	11.2	46.6	4.2	53.23	10.05	28.03	3
148	HW02061/BW781	F2	none	-	1.6	7.8	4.9	52.81	10.43	27.53	3
149	HW02061/BW781	F2	none	-	1.6	4.2	2.6	51.69	10.61	27.32	3
186	BW315a/00H4*J3	F2	none	-	29.0	69.0	2.4	52.44	9.46	26.98	2
217	HW03007/04GC0097	F2	none	1121	7.0	21.6	3.1	49.50	9.48	24.67	2
218	HW03007/04GC0177	F2	none	1347	1.4	3.2	2.3	48.50	9.24	23.54	2
219	HW02011/04GC0095	F2	none	1207	27.4	75.8	2.8	50.58	9.08	25.16	2
220	HW02011/04GC0176	F2	none	1267	2.2	6.2	2.8	49.03	9.62	23.77	2
221	HW02011/04GC0094	F2	none	1211	0.6	3.2	5.3	47.60	9.92	23.40	2
222	HW02011/04GC0097	F2	none	876	6.8	18.2	2.7	48.99	9.32	23.90	2
223	HW02011/04GC0113	F2	none	1448	1.6	7.8	4.9	49.14	9.31	23.27	2
224	HW02025/04GC0098	F2	none	1023	9.0	28.6	3.2	48.94	9.75	24.77	2
225	HW02025/04GC0095	F2	none	1340	27.6	72.4	2.6	51.06	9.21	25.61	2
226	HW02025/04GC0143	F2	none	1089	5.8	14.4	2.5	48.74	8.96	23.63	2
227	HW02025/04GC0113	F2	none	1356	0.8	2.2	2.8	49.15	9.00	23.26	2
228	HW206/04GC0143	F2	none	1471	6.2	13.4	2.2	49.96	9.77	25.30	2
229	HW0206/04GC0177	F2	none	1520	1.6	8.0	5.0	49.94	10.01	25.26	3
230	HW0206/04GC0137	F2	none	1439	4.6	14.4	3.1	49.68	10.12	26.80	3
231	HW02061/04GC0176	F2	none	1876	2.4	9.0	3.8	49.32	10.15	25.89	3
232	BW315a/04GC0098	F2	none	1605	5.2	18.6	3.6	49.21	9.78	25.09	2
233	BW315a/Alsen	F2	none	1502	2.0	6.8	3.4	49.38	10.19	25.65	3
234	BW315a/04GC0113	F2	none	1522	1.4	4.6	3.3	49.31	9.43	24.01	2
235	BW315a/04GC0176	F2	none	1667	0.6	5.2	8.7	49.23	10.01	24.89	2
239	Alsen/BW356	F3	Spring 06	1154	16.6	41.8	2.5	50.95	9.28	25.44	2
240	Alsen//BW275/HW02025	F3	Spring 06	1276	4.8	31.2	6.5	48.21	9.64	24.63	2
241	Alsen//BW275/HW02025	F3	Spring 06	1240	9.0	23.0	2.6	49.27	9.81	25.52	2

242	Alsen//BW275/HW02025	F3	Spring 06	1097	9.0	25.4	2.8	48.54	9.38	24.53	2
243	Alsen//BW275/HW02025	F3	Spring 06	1118	7.0	21.4	3.1	48.06	9.25	24.18	2
244	Alsen//BW264/HW02025	F3	Spring 06	1096	8.8	24.8	2.8	49.61	9.68	25.18	2
245	Alsen//BW264/HW02025	F3	Spring 06	1013	8.8	23.2	2.6	50.54	9.69	25.84	3
246	Alsen//BW264/HW02025	F3	Spring 06	1123	13.2	35.2	2.7	51.72	9.87	26.88	3
247	BW356/Alsen	F3	Spring 06	979	35.0	76.4	2.2	53.07	9.16	27.40	4
248	BW356/BW337	F3	Spring 06	1202	14.8	50.2	3.4	53.18	9.19	26.29	4
249	BW356/BW809	F3	Spring 06	1155	22.8	66.4	2.9	53.81	9.11	26.93	3
250	BW356/BW809	F3	Spring 06	1094	28.6	66.2	2.3	54.69	9.05	27.06	3
251	BW356/BW809	F3	Spring 06	1139	30.4	74.4	2.4	54.94	9.14	27.77	3
252	BW315a/Alsen	F3	Spring 06	1028	25.2	79.2	3.1	53.13	8.77	27.58	2
253	BW315a/BW343	F3	Spring 06	939	30.8	70.6	2.3	52.07	9.02	26.03	2
254	BW315a//W99171/X02.229	F3	Spring 06	1006	22.2	57.2	2.6	52.92	9.27	27.83	3
255	BW315a//W99171/X02.229	F3	Spring 06	947	22.8	47.0	2.1	52.96	9.17	26.93	3
256	BW315a//W99171/X02.229	F3	Spring 06	1035	17.0	59.0	3.5	53.91	9.09	27.79	3
257	BW315a//W99171/X02.229	F3	Spring 06	990	19.4	53.0	2.7	53.57	9.64	27.94	2
258	BW315a/DH74	F3	Spring 06	1027	95.8	99.6	1.0	56.12	8.61	28.83	2
259	BW315a/DH74	F3	Spring 06	937	98.0	100.0	1.0	54.44	9.06	29.40	2
260	BW315a/DH74	F3	Spring 06	925	94.6	100.0	1.1	55.11	8.98	28.84	2
261	BW315a/DH74	F3	Spring 06	947	96.4	100.0	1.0	54.70	8.76	28.34	2
262	W02504/Superb	F3	Spring 06	1416	15.4	56.2	3.6	52.53	9.53	27.92	2
263	W02504/Superb	F3	Spring 06	1185	14.8	41.8	2.8	50.44	9.93	27.70	3
264	W02504/Superb	F3	Spring 06	1261	12.0	38.8	3.2	51.29	10.12	28.41	3
265	W02504/Superb	F3	Spring 06	1265	16.8	52.4	3.1	50.55	10.38	27.44	3
266	W02504/BW356/BW776	F3	Spring 06	1042	63.4	96.0	1.5	55.26	8.97	28.66	2
267	W02504/BW356/BW776	F3	Spring 06	1083	63.2	96.8	1.5	55.03	8.90	28.54	2
268	W02504/BW356/BW776	F3	Spring 06	1021	72.4	98.2	1.4	55.22	8.71	27.91	2
269	W02504/BW356/BW776	F3	Spring 06	1046	73.4	97.8	1.3	55.63	8.54	27.79	2
270	HW03003/Superb	F3	Spring 06	1284	11.6	33.0	2.8	51.54	9.62	26.63	2
271	HW03003/Superb	F3	Spring 06	1400	8.4	34.0	4.0	52.21	9.65	26.62	3
272	HW03003/Superb	F3	Spring 06	1286	8.4	26.6	3.2	50.68	10.16	26.43	3
273	HW03003/Superb	F3	Spring 06	1484	6.0	21.8	3.6	51.40	9.63	25.98	3
274	HW03003/BW356/BW776	F3	Spring 06	1333	51.2	93.4	1.8	52.82	9.18	27.99	2
275	HW03003/BW356/BW776	F3	Spring 06	1538	53.4	97.0	1.8	53.13	9.37	28.35	2

276	HW03003/BW356/BW776	F3	Spring 06	1365	50.8	95.8	1.9	54.03	9.07	28.59	2
277	HW03003/BW356/BW776	F3	Spring 06	1277	50.8	93.8	1.8	54.03	9.24	29.11	2
278	HW03007/Superb	F3	Spring 06	1513	9.2	39.2	4.3	51.07	9.80	26.66	3
279	HW03007/Superb	F3	Spring 06	1786	20.2	73.0	3.6	51.50	9.09	26.72	3
280	HW03007/Superb	F3	Spring 06	1583	18.8	77.6	4.1	53.09	9.33	27.65	3
281	HW03007/Superb	F3	Spring 06	1453	7.8	28.6	3.7	51.40	10.30	27.46	3
282	HW03007/BW356/BW776	F3	Spring 06	1347	69.6	98.2	1.4	55.88	8.80	28.96	2
283	HW03007/BW356/BW776	F3	Spring 06	1227	59.8	97.8	1.6	54.83	9.11	28.96	2
284	HW03007/BW356/BW776	F3	Spring 06	1327	59.6	97.4	1.6	54.44	8.90	28.72	2
285	HW03007/BW356/BW776	F3	Spring 06	1387	57.4	95.2	1.7	52.13	9.31	27.26	2

Table 40. Percentage of white kernels before and after color sorting and their relative increases in 2006 and 2007 harvested bulk populations of white and red parental wheat crosses.

Plot #	Field Year	Cross	Pedigree	Gn	Before Color Sorting % White	After Color Sorting % White	Relative Increase (X's)
86	2006	X05.385	BW315a*2/04GC0098	F2	32	68	2.1
87	2006	X05.386	BW315a*2/04GC0098	F2	44	94	2.1
88	2006	X05.387	BW315a*2/04GC0098	F2	61	97	1.6
89	2006	X05.388	BW315a*2/04GC0113	F2	23	73	3.2
90	2006	X05.389	BW315a*2/Alsen	F2	43	91	2.1
91	2006	X05.390	BW315a*2/Alsen	F2	30	85	2.8
92	2006	X05.391	BW315a*2/Alsen	F2	7	31	4.8
93	2006	X05.392	BW315a//HW02011/04GC0113	F2	9	58	6.5
94	2006	X05.393	BW315a//HW02025/04GC0095	F2	75	100	1.3
95	2006	X05.394	BW315a//HW02025/04GC0113	F2	36	66	1.8
96	2006	X05.395	HW02011*2/04GC0113	F2	10	87	8.5
97	2006	X05.396	HW02025*2/04GC0095	F2	61	100	1.6
98	2006	X05.396	HW02025*2/04GC0095	F2	64	100	1.6
99	2006	X05.397	HW02025*2/04GC0113	F2	32	96	3.0
100	2006	X05.397	HW02025*2/04GC0113	F2	36	98	2.7
101	2006	X05.398	HW02061*2/04GC0143	F2	9	72	7.6
102	2006	X05.399	HW02061*2/04GC0143	F2	38	94	2.5
103	2006	X05.400	HW02061*2/04GC0177	F2	79	96	1.2
104	2006	X05.501	HW02061*2/04GC0177	F2	50	97	1.9
105	2006	X05.502	HW02061*2/04GC0177	F2	20	81	4.0
106	2006	X05.503	HW02061//HW02025/04GC0095	F2	87	100	1.2
107	2006	X05.504	HW02061//HW02025/04GC0113	F2	12	74	6.1
108	2006	X05.504	HW02061//HW02025/04GC0113	F2	24	91	3.8
109	2006	X05.505	HW02061//BW315a/Alsen	F2	13	77	5.9
110	2006	X05.506	HW02061//BW315a/Alsen	F2	8	70	8.5
111	2006	X05.507	HW03007//HW02025/04GC0095	F2	82	100	1.2
112	2006	X05.507	HW03007//HW02025/04GC0095	F2	69	100	1.5
113	2006	X05.508	BW315a*2/04GC0098	F2	37	94	2.5
114	2006	X05.509	BW315a*2/04GC0113	F2	37	88	2.4
115	2006	X05.509	BW315a*2/04GC0113	F2	37	89	2.4
116	2006	X05.510	BW315a*2/04GC0176	F2	13	41	3.1
117	2006	X05.510	BW315a*2/04GC0176	F2	16	45	2.8
118	2006	X05.511	BW315a*2/Alsen	F2	16	47	2.9
119	2006	X05.511	BW315a*2/Alsen	F2	24	63	2.6
120	2006	X05.512	BW315a//HW02011/04GC0113	F2	11	62	5.8
121	2006	X05.512	BW315a//HW02011/04GC0113	F2	17	75	4.4
122	2006	X05.513	BW315a//HW02011/04GC0176	F2	13	70	5.3
123	2006	X05.514	BW315a//HW02025/04GC0095	F2	40	98	2.4
124	2006	X05.515	BW315a//HW02025/04GC0113	F2	15	69	4.6
125	2006	X05.516	BW315a//HW02061/04GC0176	F2	13	60	4.5
126	2006	X05.517	HW02011*2/04GC0113	F2	20	93	4.6
127	2006	X05.517	HW02011*2/04GC0113	F2	14	90	6.5
128	2006	X05.518	HW02011*2/04GC0176	F2	18	85	4.8
129	2006	X05.518	HW02011*2/04GC0176	F2	23	74	3.3
130	2006	X05.519	HW02011//BW315a/04GC0176	F2	29	92	3.2

131	2006	X05.519	HW02011//BW315a/04GC0176	F2	22	77	3.5
132	2006	X05.520	HW02011//HW02061/04GC0176	F2	11	74	6.6
133	2006	X05.521	HW02025*2/04GC0095	F2	69	99	1.4
134	2006	X05.521	HW02025*2/04GC0095	F2	73	100	1.4
135	2006	X05.522	HW02025*2/04GC0113	F2	12	80	6.4
136	2006	X05.522	HW02025*2/04GC0113	F2	12	87	7.0
137	2006	X05.523	HW02025//HW02061/04GC0176	F2	49	96	2.0
138	2006	X05.523	HW02025//HW02061/04GC0176	F2	47	96	2.0
139	2006	X05.524	HW02061*2/04GC0143	F2	24	81	3.3
140	2006	X05.525	HW02061*2/04GC0176	F2	39	93	2.4
141	2006	X05.525	HW02061*2/04GC0176	F2	38	98	2.6
142	2006	X05.526	HW02061*2/04GC0177	F2	42	95	2.2
143	2006	X05.527	HW02061//HW02025/04GC0095	F2	60	95	1.6
144	2006	X05.528	HW02061//HW02025/04GC0113	F2	16	99	6.1
145	2006	X05.529	HW02061//BW315a/Alsen	F2	16	41	2.5
146	2006	X05.530	HW03007//HW02025/04GC0095	F2	82	95	1.2
147	2006	X05.530	HW03007//HW02025/04GC0095	F2	66	89	1.3
148	2006	X05.531	HW03007//HW02061/04GC0176	F2	17	46	2.7
149	2006	X05.532	BW315a*2/04GC0098	F2	28	65	2.3
150	2006	X05.533	BW315a*2/04GC0113	F2	10	57	5.5
151	2006	X05.534	BW315a*2/04GC0176	F2	11	73	6.5
152	2006	X05.535	BW315a//HW02011/04GC0113	F2	19	68	3.5
765	2007	X07.14	BW315a/Glenn	F2	1	8	7.8
766	2007	X07.14	BW315a/Glenn	F2	1.8	11	5.9
767	2007	X07.16	BW315a/BW841	F2	2.8	49	17.4
768	2007	X07.16	BW315a/BW841	F2	5.4	23	4.3
769	2007	X07.17	BW315a/BW385	F2	1.8	6	3.3
770	2007	X07.18	BW315a/BW389	F2	1.4	8	5.4
771	2007	X07.18	BW315a/BW389	F2	2.2	11	5.0
772	2007	X07.18	BW315a/BW389	F2	5.8	20	3.4
773	2007	X07.19	BW315a//BW357/BW362	F2	5.2	21	4.1
774	2007	X07.20	BW315a//Lillian/BW362	F2	1.4	5	3.4
775	2007	X07.20	BW315a//Lillian/BW362	F2	2.2	7	3.0
776	2007	X07.22	BW315a/PT610	F2	3	9	2.9
777	2007	X07.22	BW315a/PT610	F2	1.6	8	5.1
799	2007	X07.36	Glenn//HW05039/HW001	F2	1.4	13	9.4
800	2007	X07.36	Glenn//HW05039/HW001	F2	1.4	7	5.3
806	2007	X07.44	Lillian//HW05039/HW05034	F2	0.8	4	4.5
825	2007	X07.57	BW841//HW05039/HW05034	F2	5.6	23	4.2
826	2007	X07.57	BW841//HW05039/HW05034	F2	7.2	22	3.1
827	2007	X07.59	BW841//HW05039/HW001	F2	4.2	17.0	4.0
843	2007	X07.66	F0071//HW05039/HW05034	F2	0.8	7.4	9.3
844	2007	X07.66	F0071//HW05039/HW05034	F2	0.8	11.2	14.0
845	2007	X07.66	F0071//HW05039/HW05034	F2	0.4	6.8	17.0
846	2007	X07.68	F0071//HW05039/HW001	F2	1.8	4.6	2.6
847	2007	X07.68	F0071//HW05039/HW001	F2	1.6	4.6	2.9
853	2007	X07.73	BW385//HW05039/HW05034	F2	1.6	7.4	4.6
854	2007	X07.73	BW385//HW05039/HW05034	F2	2.2	6.0	2.7
856	2007	X07.77	BW389//HW05039/HW05034	F2	2.6	10.6	4.1
857	2007	X07.78	BW389//HW05039/W04300	F2	4.2	14.4	3.4

858	2007	X07.78	BW389//HW05039/W04300	F2	4.0	13.8	3.5
859	2007	X07.79	BW389//HW05039/HW001	F2	1.8	10.0	5.6
860	2007	X07.79	BW389//HW05039/HW001	F2	3.0	7.2	2.4

Table 41. Percentage of white-grained spikes before and after color sorting in 2006 and percentage of white-grained spikes after harvest in 2007 for bulk populations of white x red wheat crosses.

		2006			2007	
Cross	Pedigree	Gen	% White Kernels Before Color Sorting	% White Kernels After Color Sorting	Gen	% Spikes with White Grain After Harvest
x04.54	Alsen/BW356	F3	17	42	F4	45
x04.55	Alsen//BW275/HW02025	F3	5	31	F4	45
x04.55	Alsen//BW275/HW02025	F3	9	23	F4	36
x04.55	Alsen//BW275/HW02025	F3	9	25	F4	34
x04.55	Alsen//BW275/HW02025	F3	7	21	F4	26
x04.56	Alsen//BW264/HW02025	F3	9	25	F4	30
x04.56	Alsen//BW264/HW02025	F3	9	23	F4	30
x04.56	Alsen//BW264/HW02025	F3	13	35	F4	51
x04.58	BW356/Alsen	F3	35	76	F4	79
x04.59	BW356/BW337	F3	15	50	F4	53
x04.60	BW356/BW809	F3	23	66	F4	68
x04.60	BW356/BW809	F3	29	66	F4	74
x04.60	BW356/BW809	F3	30	74	F4	71
x04.61	BW315a/Alsen	F3	25	79	F4	80
x04.62	BW315a/BW343	F3	31	71	F4	74
x04.64	BW315a//W99171/X02.229	F3	22	57	F4	61
x04.64	BW315a//W99171/X02.229	F3	23	47	F4	57
x04.64	BW315a//W99171/X02.229	F3	17	59	F4	65
x04.64	BW315a//W99171/X02.229	F3	19	53	F4	55
x04.65	BW315a/DH74	F3	96	100	F4	99.5
x04.65	BW315a/DH74	F3	98	100	F4	100
x04.65	BW315a/DH74	F3	95	100	F4	99
x04.65	BW315a/DH74	F3	96	100	F4	100
x04.97	W02504/Superb	F3	15	56	F4	56
x04.97	W02504/Superb	F3	15	42	F4	43
x04.97	W02504/Superb	F3	12	39	F4	46
x04.97	W02504/Superb	F3	17	52	F4	55
x04.99	W02504/BW356/BW776	F3	63	96	F4	94
x04.99	W02504/BW356/BW776	F3	63	97	F4	95
x04.99	W02504/BW356/BW776	F3	72	98	F4	96
x04.99	W02504/BW356/BW776	F3	73	98	F4	98
x04.102	HW03003/Superb	F3	12	33	F4	42
x04.102	HW03003/Superb	F3	8	34	F4	35
x04.102	HW03003/Superb	F3	8	27	F4	26
x04.102	HW03003/Superb	F3	6	22	F4	31
x04.104	HW03003/BW356/BW776	F3	51	93	F4	90
x04.104	HW03003/BW356/BW776	F3	53	97	F4	96
x04.104	HW03003/BW356/BW776	F3	51	96	F4	95
x04.104	HW03003/BW356/BW776	F3	51	94	F4	92
x04.107	HW03007/Superb	F3	9	39	F4	46
x04.107	HW03007/Superb	F3	20	73	F4	78
x04.107	HW03007/Superb	F3	19	78	F4	78

x04.107	HW03007/Superb	F3	8	29	F4	32
x04.108	HW03007/BW356/BW776	F3	70	98	F4	96
x04.108	HW03007/BW356/BW776	F3	60	98	F4	94
x04.108	HW03007/BW356/BW776	F3	60	97	F4	96
x04.108	HW03007/BW356/BW776	F3	57	95	F4	95

Table 42. Percentage of white grain before and after color sorting in each of the 2007 F_3 bulk populations segregating for red and white grain.

			2006		2007	
2007 Plot	Cross	Pedigree	Before % Whites	After % Whites	Before % Whites	After % Whites
317	x06.152	HW04300/06HWFHBX1	-*	-	78	100
318	x06.153	HW04300/06HWFHBX2	-	-	100	100
319	x06.153	HW04300/06HWFHBX2	-	-	72	98
320	x06.155	HW04300/06HWFHBX3	-	-	96	100
321	x06.155	HW04300/06HWFHBX3	-	-	98	100
322	x06.156	HW04300/06HWFHBX4	-	-	80	100
323	x06.157	HW04300/06HWFHBX6	-	-	92	100
324	x06.157	HW04300/06HWFHBX6	-	-	82	98
325	x06.158	HW04300/06HWFHBX8	-	-	100	100
326	x06.158	HW04300/06HWFHBX8	-	-	96	100
327	x06.159	HW04300/06HWFHBX10	-	-	86	96
328	x06.161	HW04300/06HWFHBX18	-	-	76	98
329	x06.161	HW04300/06HWFHBX18	-	-	72	96
330	x06.162	HW05034/06HWFHBX1	-	-	88	98
331	x06.162	HW05034/06HWFHBX1	-	-	96	100
332	x06.163	HW05034/06HWFHBX2	-	-	94	100
333	x06.163	HW05034/06HWFHBX2	-	-	94	100
334	x06.164	HW05034/06HWFHBX3	-	-	98	100
335	x06.165	HW05034/06HWFHBX3	-	-	94	100
336	x06.165	HW05034/06HWFHBX3	-	-	94	100
337	x06.166	HW05034/06HWFHBX4	-	-	92	98
338	x06.166	HW05034/06HWFHBX4	-	-	82	100
339	x06.167	HW05034/06HWFHBX6	-	-	56	82
340	x06.167	HW05034/06HWFHBX6	-	-	68	92
341	x06.168	HW05034/06HWFHBX8	-	-	94	100
342	x06.168	HW05034/06HWFHBX8	-	-	94	98
343	x06.169	HW05034/06HWFHBX10	-	-	64	94
344	x06.170	HW05034/06HWFHBX12	-	-	72	96
345	x06.171	HW05034/06HWFHBX13	-	-	68	98
346	x06.171	HW05034/06HWFHBX13	-	-	78	94
347	x06.172	HW05034/06HWFHBX18	-	-	82	98
348	x06.172	HW05034/06HWFHBX18	-	-	76	98
349	x06.173	HW05034/06HWFHBX23	-	-	64	96
350	x06.174	HW05034/06HWFHBX26	-	-	70	96
351	x06.174	HW05034/06HWFHBX26	-	-	68	90
352	x06.175	HW05039/06HWFHBX1	-	-	94	100
353	x06.175	HW05039/06HWFHBX1	-	-	90	98

354	x06.176	HW05039/06HWFHBX2	-	-	54	92
355	x06.176	HW05039/06HWFHBX2	-	-	58	94
356	x06.177	HW05039/06HWFHBX3	-	-	84	96
357	x06.177	HW05039/06HWFHBX3	-	-	90	98
358	x06.178	HW05039/06HWFHBX4	-	-	88	94
359	x06.178	HW05039/06HWFHBX4	-	-	88	98
360	x06.179	HW05039/06HWFHBX6	-	-	72	78
361	x06.179	HW05039/06HWFHBX6	-	-	46	98
362	x06.180	HW05039/06HWFHBX8	-	-	96	98
363	x06.180	HW05039/06HWFHBX8	-	-	94	100
364	x06.181	HW05039/06HWFHBX10	-	-	72	90
365	x06.182	HW05039/06HWFHBX12	-	-	68	96
366	x06.183	HW05039/06HWFHBX13	-	-	72	96
367	x06.183	HW05039/06HWFHBX13	-	-	58	92
368	x06.185	HW05039/06HWFHBX23	-	-	66	84
369	x06.185	HW05039/06HWFHBX23	-	-	48	92
453	x06.154	HW04300/06HWFHBX3	-	-	98	100
454	x06.160	HW04300/06HWFHBX12	-	-	50	74
455	x06.184	HW05039/06HWFHBX18	-	-	80	98
457	x06.228	HW04028//BW315a/4/HW03007/3/W02504/BW356	-	-	98	100
458	X05.240	HW03007/04GC0097	7	22	28	64
459	X05.241	HW03007/04GC0177	1	3	18	38
460	X05.242	HW02011/04GC0095	27	76	18	96
461	X05.243	HW02011/04GC0176	2	6	12	32
462	X05.244	HW02011/04GC0094	1	3	2	40
463	X05.245	HW02011/04GC0097	7	18	26	50
464	X05.246	HW02011/04GC0113	2	8	6	18
465	X05.247	HW02025/04GC0098	9	29	26	62
466	X05.248	HW02025/04GC0095	28	72	74	94
467	X05.249	HW02025/04GC0143	6	14	24	60
468	X05.250	HW02025/04GC0113	1	2	6	12
469	X05.251	HW2061/04GC0143	6	13	22	44
470	X05.252	HW02061/04GC0177	2	8	10	54
471	X05.253	HW02061/04GC0137	5	14	22	66
472	X05.254	HW02061/04GC0176	2	9	12	68
473	X05.255	BW315a/04GC0098	5	19	16	58
474	X05.256	BW315a/Alsen	2	7	10	26
475	X05.257	BW315a/04GC0113	1	5	16	26
476	X05.258	BW315a/04GC0176	1	5	16	40
932	X05.388	05HWFHBX2	23	73	70	98
933	X05.390	05HWFHBX4	30	85	80	94
934	X05.392	05HWFHBX5	9	58	60	100
935	X05.395	05HWFHBX10	10	87	90	100
936	X05.396	05HWFHBX14	63	100	98	100
937	X05.397	05HWFHBX15	34	97	98	100
938	X05.504	05HWFHBX21	18	83	76	100
940	X05.508	05HWFHBX1	37	94	82	96
941	X05.509	05HWFHBX2	37	89	98	100

942	X05.510	05HWFHBX3	14	43	56	90
943	X05.511	05HWFHBX4	20	55	58	92
944	X05.512	05HWFHBX5	14	69	86	96
945	X05.513	05HWFHBX6	13	70	76	94
946	X05.514	05HWFHBX7	40	98	96	98
947	X05.515	05HWFHBX8	15	69	78	94
948	X05.516	05HWFHBX9	13	60	60	100
949	X05.517	05HWFHBX10	17	92	92	100
950	X05.518	05HWFHBX11	20	80	90	98
951	X05.519	05HWFHBX12	26	85	80	100
952	X05.520	05HWFHBX13	11	74	80	98
953	X05.521	05HWFHBX14	71	99	100	100
954	X05.522	05HWFHBX15	12	83	78	100
955	X05.523	05HWFHBX16	48	96	100	100
956	X05.524	05HWFHBX17	24	81	78	94
957	X05.525	05HWFHBX18	38.5	96	100	100
958	X05.526	05HWFHBX19	42	95	98	100
959	X05.527	05HWFHBX20	60	95	94	98
960	X05.528	05HWFHBX21	16	99	72	100
961	X05.529	05HWFHBX22	16	41	56	84
962	X05.530	05HWFHBX23	74	92	94	98
963	X05.531	05HWFHBX24	17	46	90	96
964	X05.532	05HWFHBX1	28	65	90	92
965	X05.533	05HWFHBX2	10	57	64	92
966	X05.534	05HWFHBX3	11	73	82	100
967	X05.535	05HWFHBX5	19	68	72	96
968	X05.385	BW315a*2/04GC0098	32	68	78	92
969	X05.386	BW315a*2/04GC0098	44	94	94	98
970	X05.387	BW315a*2/04GC0098	61	97	96	100
971	X05.389	BW315a*2/Alsen	43	91	94	100
972	X05.391	BW315a*2/Alsen	7	31	50	92
973	X05.393	BW315a//HW02025/04GC0095	75	100	96	100
974	X05.394	BW315a//HW02025/04GC0113	36	66	76	100
976	X05.399	HW02061*2/04GC0143	38	94	96	98
977	X05.400	HW02061*2/04GC0177	79	96	100	100
978	X05.501	HW02061*2/04GC0177	50	97	100	100
979	X05.502	HW02061*2/04GC0177	20	81	84	98
980	X05.503	HW02061//HW02025/04GC0095	87	100	100	100
981	X05.505	HW02061//BW315a/Alsen	13	77	82	100
982	X05.507	HW03007//HW02025/04GC0095	82	100	98	100
983	X05.507	HW03007//HW02025/04GC0095	69	100	100	100
1032	X05.573	06HWFHBX1	-	-	66	86
1033	X05.577	06HWFHBX2	-	-	62	86
1034	X05.580	06HWFHBX2	-	-	32	82
1035	X05.581	06HWFHBX3	-	-	100	100
1036	X05.584	06HWFHBX3	-	-	100	100
1037	X05.588	06HWFHBX4	-	-	58	98
1038	X05.589	06HWFHBX5	-	-	56	100

1039	X05.591	06HWFHBX6	-	-	30	92
1040	X05.592	06HWFHBX6	-	-	52	94
1041	X05.593	06HWFHBX6	-	-	84	100
1042	X05.594	06HWFHBX6	-	-	54	98
1043	X05.595	06HWFHBX7	-	-	68	100
1044	X05.596	06HWFHBX7	-	-	58	98
1045	X05.598	06HWFHBX8	-	-	100	100
1046	X05.599	06HWFHBX8	-	-	68	96
1047	X05.600	06HWFHBX8	-	-	80	100
1048	X05.601	06HWFHBX8	-	-	100	100
1049	X05.603	06HWFHBX9	-	-	68	98
1050	X05.604	06HWFHBX9	-	-	44	100
1051	X05.608	06HWFHBX10	-	-	62	100
1052	X05.609	06HWFHBX11	-	-	100	100
1053	X05.610	06HWFHBX11	-	-	98	100
1054	X05.611	06HWFHBX11	-	-	100	100
1055	X05.613	06HWFHBX12	-	-	66	100
1056	X05.614	06HWFHBX12	-	-	78	96
1057	X05.616	06HWFHBX13	-	-	38	94
1058	X05.618	06HWFHBX13	-	-	66	100
1059	X05.620	06HWFHBX13	-	-	66	90
1060	X05.622	06HWFHBX14	-	-	100	100
1061	X05.623	06HWFHBX14	-	-	100	100
1062	X05.624	06HWFHBX15	-	-	40	94
1063	X05.628	06HWFHBX17	-	-	100	100
1064	X05.629	06HWFHBX18	-	-	20	84
1065	X05.632	06HWFHBX19	-	-	26	92
1066	X05.635	06HWFHBX20	-	-	16	84
1067	X05.636	06HWFHBX21	-	-	42	82
1068	X05.638	06HWFHBX21	-	-	96	100
1069	X05.640	06HWFHBX22	-	-	44	100
1070	X05.641	06HWFHBX22	-	-	74	98
1071	X05.642	06HWFHBX23	-	-	20	70
1072	X05.643	06HWFHBX23	-	-	4	16
1073	X05.646	06HWFHBX24	-	-	52	76
1074	X05.648	06HWFHBX25	-	-	64	92
1075	X05.651	06HWFHBX26	-	-	16	54
1076	X05.652	06HWFHBX26	-	-	46	92

* - No previous color sorting records for this cross

Table 43. Frequency of white grain versus red grain in segregating populations obtained from plots following color sort with the Satake ScanMasterII. Seed was obtained from plots (100 heads) which had been sorted prior to seeding dry as well as after application of NaOH to enhance color differentiation.

			Frequency								
2008		2009	Dry Sort		NaOH Sort		2008 Reserve Color Sort NaOH				NaOH White
Plot	Cross	Plot	White	Red	White	Red	White	Red	%White	% Red	Increase
1072	X08.47	577/977	57	43	21	79	21	479	4	96	5.0
1073	X08.47	578/978	81	19	45	54	27	473	5	95	8.3
1074	X08.48	579/979	56	44	18	82	23	477	5	95	3.9
1075	X08.48	580/980	64	36	26	74	23	477	5	95	5.7
1076	X08.49	581/981	86	14	34	66	33	467	7	93	5.2
1077	X08.49	582/982	69	31	25	75	26	474	5	95	4.8
1078	X08.50	583/983	75	25	34	66	25	475	5	95	6.8
1079	X08.50	584/984	31	69	15	85	13	487	3	97	5.8
1080	X08.51	585/985	59	41	41	69	40	460	8	92	5.1
1081	X08.51	586/986	58	42	21	79	55	445	11	89	1.9
1082	X08.51	587/987	61	39	29	71	29	471	6	94	5.0
1083	X08.52	588/988	75	25	37	63	37	463	7	93	5.0
1084	X08.52	589/989	50	50	15	85	27	473	5	95	2.8
1085	X08.53	590/990	56	44	40	60	39	461	8	92	5.1
Average			62.7	37.3	28.6	72.0	29.9	470.1	6.0	94.0	5.0

Table 44. Parental FHB marker classification				
	3BS	3BS	5AS	5AS
	gwm533	gwm493	gwm304	gwm293
BW315a	S	S	S	S
HW02011	S	S	S	S
HW02025	S	S	S	S
HW02061	S	S	S	S
HW03007	S	S	S	S
Alsen	R	S	R	R
04GC0094	R	R	S	S
04GC0095	R	R	S	S
04GC00139	R	R	R	R
04GC00177	R	R	R	R
04GC00113	R	S	S	S
04GC00137	R	R	R	R
04GC0098	R	R	R	R
04GC0097	R	R	S	S
04GC00143	R	R	R	R

			3BS	3BS	5AS	5AS
Cross 05#	Pedigree	n	gwm533	gwm493	gwm304	gwm293
HWfhbX1	BW315a*2/04GC0098	4	plus	plus	plus	plus
HWfhbX1	BW315a*2/04GC0098	4	plus	plus	minus	minus
HWfhbX1	BW315a*2/04GC0098	5	minus	minus	plus	plus
HWfhbX1	BW315a*2/04GC0098	8	minus	minus	minus	minus
HWfhbX1	BW315a*2/04GC0098	17	plus	minus	minus	minus
HWfhbX2	BW315a*2/04GC0113	31	minus	minus	minus	minus
HWfhbX2	BW315a*2/04GC0113	12	plus	minus	plus	plus
HWfhbX4	BW315a*2/Alsen	17	plus	minus	minus	minus
HWfhbX4	BW315a*2/Alsen	16	minus	minus	plus	plus
HWfhbX4	BW315a*2/Alsen	9	minus	minus	minus	minus
HWfhbX4	BW315a*2/Alsen	14	plus	minus	minus	minus
HWfhbX5	BW315a//HW02011/04GC0113	34	minus	minus	minus	minus
HWfhbX5	BW315a//HW02011/04GC0113	7	plus	plus	minus	minus
HWfhbX7	BW315a//HW02025/04GC0095	11	minus	minus	minus	minus
HWfhbX7	BW315a//HW02025/04GC0095	5	plus	minus	minus	minus
HWfhbX8	BW315a//HW02025/04GC0113	7	minus	minus	minus	minus
HWfhbX8	BW315a//HW02025/04GC0113	8	plus	minus	minus	minus
HWfhbX10	HW02011*2/04GC0113	22	minus	minus	minus	minus
HWfhbX10	HW02011*2/04GC0113	12	plus	plus	minus	minus
HWfhbX14	HW02025*2/04GC0095	27	minus	minus	minus	minus
HWfhbX14	HW02025*2/04GC0095	34	plus	minus	minus	minus
HWfhbX15	HW02025*2/04GC0113	35	minus	minus	minus	minus
HWfhbX15	HW02025*2/04GC0113	10	plus	minus	minus	minus
HWfhbX17	HW02061*2/04GC0143 (133?)	14	minus	minus	minus	minus

HWfhbX17	HW02061*2/04GC0143 (133?)	2	plus	plus	plus	plus
HWfhbX19	HW02061*2/04GC0177	4	plus	plus	minus	minus
HWfhbX19	HW02061*2/04GC0177	5	minus	minus	plus	plus
HWfhbX19	HW02061*2/04GC0177	7	minus	minus	minus	minus
HWfhbX19	HW02061*2/04GC0177	6	plus	plus	minus	minus
HWfhbX20	HW02061//HW02025/04GC0095	9	minus	minus	minus	minus
HWfhbX20	HW02061//HW02025/04GC0095	15	plus	minus	minus	minus
HWfhbX21	HW02061//HW02025/04GC0113	9	minus	minus	minus	minus
HWfhbX21	HW02061//HW02025/04GC0113	2	plus	minus	plus	plus
HWfhbX22	HW02061//BW315a/Alsen	1	plus	minus	minus	minus
HWfhbX22	HW02061//BW315a/Alsen	1	minus	minus	plus	plus
HWfhbX22	HW02061//BW315a/Alsen	5	minus	minus	minus	minus
HWfhbX22	HW02061//BW315a/Alsen	20	plus	plus	minus	minus
HWfhbX23	HW03007//HW02025/04GC0095	16	minus	minus	minus	minus
	Total number of seedlings screened:	465				

Table 45. Hard White wheat populations genotyped for the 3BS and 5AS fhb QTL markers in 2006

2006 Plot #	Cross	reference #	Pedigree	2006 Gn	F1 plant FHB mk	destination
86	X05.385	05HWFHBX1	BW315a*2/04GC0098	F2	++++nn	06/07NZ
87	X05.386	05HWFHBX1	BW315a*2/04GC0098	F2	++--nn	06/07NZ
88	X05.387	05HWFHBX1	BW315a*2/04GC0098	F2	--++nn	06/07NZ
89	X05.388	05HWFHBX2	BW315a*2/04GC0113	F2	+nnn??	2007 bulk
90	X05.389	05HWFHBX4	BW315a*2/Alsen	F2	+n++??	06/07NZ
91	X05.390	05HWFHBX4	BW315a*2/Alsen	F2	+n--??	2007 bulk
92	X05.391	05HWFHBX4	BW315a*2/Alsen	F2	-n++??	06/07NZ
93	X05.392	05HWFHBX5	BW315a//HW02011/04GC0113	F2	+nnn??	2007 bulk
94	X05.393	05HWFHBX7	BW315a//HW02025/04GC0095	F2	++nn??	06/07NZ
95	X05.394	05HWFHBX8	BW315a//HW02025/04GC0113	F2	+nnn??	06/07NZ
96	X05.395	05HWFHBX10	HW02011*2/04GC0113	F2	+nnn??	2007 bulk
97	X05.396	05HWFHBX14	HW02025*2/04GC0095	F2	++nn??	2007 bulk
98	X05.396	05HWFHBX14	HW02025*2/04GC0095	F2	++nn??	2007 bulk
99	X05.397	05HWFHBX15	HW02025*2/04GC0113	F2	+nnn??	2007 bulk
100	X05.397	05HWFHBX15	HW02025*2/04GC0113	F2	+nnn??	2007 bulk
101	X05.398	05HWFHBX17	HW02061*2/04GC0143	F2	++++??	06/07NZ
102	X05.399	05HWFHBX17	HW02061*2/04GC0143	F2	++--??	06/07NZ
103	X05.400	05HWFHBX19	HW02061*2/04GC0177	F2	++++?n	06/07NZ
104	X05.501	05HWFHBX19	HW02061*2/04GC0177	F2	++--?n	06/07NZ
105	X05.502	05HWFHBX19	HW02061*2/04GC0177	F2	--++n?	06/07NZ
106	X05.503	05HWFHBX20	HW02061//HW02025/04GC0095	F2	++nn??	06/07NZ
107	X05.504	05HWFHBX21	HW02061//HW02025/04GC0113	F2	+nnn??	2007 bulk
108	X05.504	05HWFHBX21	HW02061//HW02025/04GC0113	F2	+nnn??	2007 bulk
109	X05.505	05HWFHBX22	HW02061//BW315a/Alsen	F2	+n++??	06/07NZ
110	X05.506	05HWFHBX22	HW02061//BW315a/Alsen	F2	+n--??	2007 bulk
111	X05.507	05HWFHBX23	HW03007//HW02025/04GC0095	F2	++nn??	06/07NZ
112	X05.507	05HWFHBX23	HW03007//HW02025/04GC0095	F2	++nn??	06/07NZ
113	X05.508	05HWFHBX1	BW315a*2/04GC0098	F2	n bulk	2007 bulk
114	X05.509	05HWFHBX2	BW315a*2/04GC0113	F2	n? bulk	2007 bulk
115	X05.509	05HWFHBX2	BW315a*2/04GC0113	F2	n? bulk	2007 bulk
116	X05.510	05HWFHBX3	BW315a*2/04GC0176	F2	n? bulk	2007 bulk
117	X05.510	05HWFHBX3	BW315a*2/04GC0176	F2	n? bulk	2007 bulk
118	X05.511	05HWFHBX4	BW315a*2/Alsen	F2	n? bulk	2007 bulk
119	X05.511	05HWFHBX4	BW315a*2/Alsen	F2	n? bulk	2007 bulk
120	X05.512	05HWFHBX5	BW315a//HW02011/04GC0113	F2	n? bulk	2007 bulk
121	X05.512	05HWFHBX5	BW315a//HW02011/04GC0113	F2	n? bulk	2007 bulk
122	X05.513	05HWFHBX6	BW315a//HW02011/04GC0176	F2	n? bulk	2007 bulk
123	X05.514	05HWFHBX7	BW315a//HW02025/04GC0095	F2	n? bulk	2007 bulk

124	X05.515	05HWFHBX8	BW315a//HW02025/04GC0113	F2	n? bulk	2007 bulk
125	X05.516	05HWFHBX9	BW315a//HW02061/04GC0176	F2	n? bulk	2007 bulk
126	X05.517	05HWFHBX10	HW02011*2/04GC0113	F2	n? bulk	2007 bulk
127	X05.517	05HWFHBX10	HW02011*2/04GC0113	F2	n? bulk	2007 bulk
128	X05.518	05HWFHBX11	HW02011*2/04GC0176	F2	n? bulk	2007 bulk
129	X05.518	05HWFHBX11	HW02011*2/04GC0176	F2	n? bulk	2007 bulk
130	X05.519	05HWFHBX12	HW02011//BW315a/04GC0176	F2	n? bulk	2007 bulk
131	X05.519	05HWFHBX12	HW02011//BW315a/04GC0176	F2	n? bulk	2007 bulk
132	X05.520	05HWFHBX13	HW02011//HW02061/04GC0176	F2	n? bulk	2007 bulk
133	X05.521	05HWFHBX14	HW02025*2/04GC0095	F2	n? bulk	2007 bulk
134	X05.521	05HWFHBX14	HW02025*2/04GC0095	F2	n? bulk	2007 bulk
135	X05.522	05HWFHBX15	HW02025*2/04GC0113	F2	n? bulk	2007 bulk
136	X05.522	05HWFHBX15	HW02025*2/04GC0113	F2	n? bulk	2007 bulk
137	X05.523	05HWFHBX16	HW02025//HW02061/04GC0176	F2	n? bulk	2007 bulk
138	X05.523	05HWFHBX16	HW02025//HW02061/04GC0176	F2	n? bulk	2007 bulk
139	X05.524	05HWFHBX17	HW02061*2/04GC0143	F2	n? bulk	2007 bulk
140	X05.525	05HWFHBX18	HW02061*2/04GC0176	F2	n? bulk	2007 bulk
141	X05.525	05HWFHBX18	HW02061*2/04GC0176	F2	n? bulk	2007 bulk
142	X05.526	05HWFHBX19	HW02061*2/04GC0177	F2	n? bulk	2007 bulk
143	X05.527	05HWFHBX20	HW02061//HW02025/04GC0095	F2	n? bulk	2007 bulk
144	X05.528	05HWFHBX21	HW02061//HW02025/04GC0113	F2	n? bulk	2007 bulk
145	X05.529	05HWFHBX22	HW02061//BW315a/Alsen	F2	n? bulk	2007 bulk
146	X05.530	05HWFHBX23	HW03007//HW02025/04GC0095	F2	n? bulk	2007 bulk
147	X05.530	05HWFHBX23	HW03007//HW02025/04GC0095	F2	n? bulk	2007 bulk
148	X05.531	05HWFHBX24	HW03007//HW02061/04GC0176	F2	n? bulk	2007 bulk
149	X05.532	05HWFHBX1	BW315a*2/04GC0098	F2	no geno	2007 bulk
150	X05.533	05HWFHBX2	BW315a*2/04GC0113	F2	no geno	2007 bulk
151	X05.534	05HWFHBX3	BW315a*2/04GC0176	F2	no geno	2007 bulk
152	X05.535	05HWFHBX5	BW315a//HW02011/04GC0113	F2	no geno	2007 bulk
203	X05.573	06HWFHBX1	BW315a/05HWFHBX1++++nn	F2	----nn	2007 bulk
204	X05.573	06HWFHBX1	BW315a/05HWFHBX1++++nn	F2	----nn	2007 bulk
205	X05.574	06HWFHBX1	BW315a/05HWFHBX1++++nn	F2	++--nn	06/07NZ
206	X05.574	06HWFHBX1	BW315a/05HWFHBX1++++nn	F2	++--nn	06/07NZ
207	X05.575	06HWFHBX1	BW315a/05HWFHBX1++++nn	F2	++++nn	AGGH SSD
208	X05.575	06HWFHBX1	BW315a/05HWFHBX1++++nn	F2	++++nn	AGGH SSD
209	X05.576	06HWFHBX1	BW315a/05HWFHBX1++++nn	F2	--++nn	06/07NZ
210	X05.576	06HWFHBX1	BW315a/05HWFHBX1++++nn	F2	--++nn	06/07NZ
211	X05.577	06HWFHBX2	BW315a/05HWFHBX4+-++??	F2	-n---?	2007 bulk
212	X05.577	06HWFHBX2	BW315a/05HWFHBX4+-++??	F2	-n---?	2007 bulk
213	X05.578	06HWFHBX2	BW315a/05HWFHBX4+-++??	F2	+n++-?	AGGH SSD
214	X05.579	06HWFHBX2	BW315a/05HWFHBX4+-++??	F2	-n++-?	06/07NZ
215	X05.580	06HWFHBX2	BW315a/05HWFHBX4+-++??	F2	+n---?	2007 bulk
216	X05.581	06HWFHBX3	BW315a/05HWFHBX7++--??	F2	--nn+?	2007 bulk
217	X05.581	06HWFHBX3	BW315a/05HWFHBX7++--??	F2	--nn+?	2007 bulk
218	X05.582	06HWFHBX3	BW315a/05HWFHBX7++--??	F2	++nn+?	AGGH SSD

219	X05.583	06HWFHBX3	BW315a/05HWFHBX7++--??	F2	++nn-?	06/07NZ
220	X05.584	06HWFHBX3	BW315a/05HWFHBX7++--??	F2	--nn-?	2007 bulk
221	X05.584	06HWFHBX3	BW315a/05HWFHBX7++--??	F2	--nn-?	2007 bulk
222	X05.585	06HWFHBX4	HW02011/05HWFHBX1++++nn	F2	--++nn	06/07NZ
223	X05.586	06HWFHBX4	HW02011/05HWFHBX1++++nn	F2	++--nn	06/07NZ
224	X05.586	06HWFHBX4	HW02011/05HWFHBX1++++nn	F2	++--nn	06/07NZ
225	X05.587	06HWFHBX4	HW02011/05HWFHBX1++++nn	F2	++++nn	AGGH SSD
226	X05.588	06HWFHBX4	HW02011/05HWFHBX1++++nn	F2	----nn	2007 bulk
227	X05.588	06HWFHBX4	HW02011/05HWFHBX1++++nn	F2	----nn	2007 bulk
228	X05.589	06HWFHBX5	HW02011/05HWFHBX1++--nn	F2	--nnnn	2007 bulk
229	X05.589	06HWFHBX5	HW02011/05HWFHBX1++--nn	F2	--nnnn	2007 bulk
230	X05.590	06HWFHBX5	HW02011/05HWFHBX1++--nn	F2	++nnnn	06/07NZ
231	X05.591	06HWFHBX6	HW02011/05HWFHBX4+-++??	F2	+n++-?	2007 bulk
232	X05.592	06HWFHBX6	HW02011/05HWFHBX4+-++??	F2	+n---?	2007 bulk
233	X05.592	06HWFHBX6	HW02011/05HWFHBX4+-++??	F2	+n---?	2007 bulk
234	X05.593	06HWFHBX6	HW02011/05HWFHBX4+-++??	F2	-n++-?	2007 bulk
235	X05.594	06HWFHBX6	HW02011/05HWFHBX4+-++??	F2	-n---?	2007 bulk
236	X05.594	06HWFHBX6	HW02011/05HWFHBX4+-++??	F2	-n---?	2007 bulk
237	X05.595	06HWFHBX7	HW02025/05HWFHBX4+-++??	F2	+n---?	2007 bulk
238	X05.596	06HWFHBX7	HW02025/05HWFHBX4+-++??	F2	-n---?	2007 bulk
239	X05.597	06HWFHBX7	HW02025/05HWFHBX4+-++??	F2	-n++-?	06/07NZ
240	X05.598	06HWFHBX8	HW02025/05HWFHBX14++--??	F2	++nn+?	2007 bulk
241	X05.599	06HWFHBX8	HW02025/05HWFHBX14++--??	F2	++nn-?	2007 bulk
242	X05.600	06HWFHBX8	HW02025/05HWFHBX14++--??	F2	--nn-?	2007 bulk
243	X05.600	06HWFHBX8	HW02025/05HWFHBX14++--??	F2	--nn-?	2007 bulk
244	X05.601	06HWFHBX8	HW02025/05HWFHBX14++--??	F2	--nn+?	2007 bulk
245	X05.602	06HWFHBX9	HW02061/05HWFHBX14++--??	F2	++nn-?	06/07NZ
246	X05.603	06HWFHBX9	HW02061/05HWFHBX14++--??	F2	--nn-?	2007 bulk
247	X05.604	06HWFHBX9	HW02061/05HWFHBX14++--??	F2	--nn+?	2007 bulk
248	X05.605	06HWFHBX10	HW02061/05HWFHBX19++++??	F2	++---?	06/07NZ
249	X05.606	06HWFHBX10	HW02061/05HWFHBX19++++??	F2	--++-?	06/07NZ
250	X05.607	06HWFHBX10	HW02061/05HWFHBX19++++??	F2	++++-?	AGGH SSD
251	X05.608	06HWFHBX10	HW02061/05HWFHBX19++++??	F2	-----?	2007 bulk
252	X05.609	06HWFHBX11	HW02061/05HWFHBX20++--??	F2	+nnn-?	2007 bulk
253	X05.610	06HWFHBX11	HW02061/05HWFHBX20++--??	F2	-nnn-?	2007 bulk
254	X05.611	06HWFHBX11	HW02061/05HWFHBX20++--??	F2	-nnn+?	2007 bulk
255	X05.612	06HWFHBX12	HW02061/05HWFHBX22+-++??	F2	-n++-?	06/07NZ
256	X05.613	06HWFHBX12	HW02061/05HWFHBX22+-++??	F2	-n---?	2007 bulk
257	X05.614	06HWFHBX12	HW02061/05HWFHBX22+-++??	F2	+n---?	2007 bulk
258	X05.615	06HWFHBX12	HW02061/05HWFHBX22+-++??	F2	+n++-?	AGGH SSD
259	X05.616	06HWFHBX13	HW03007/05HWFHBX4+-++??	F2	-n--+?	2007 bulk
260	X05.617	06HWFHBX13	HW03007/05HWFHBX4+-++??	F2	+n++-?	AGGH SSD
261	X05.618	06HWFHBX13	HW03007/05HWFHBX4+-++??	F2	+n---?	2007 bulk
262	X05.618	06HWFHBX13	HW03007/05HWFHBX4+-++??	F2	+n---?	2007 bulk
263	X05.619	06HWFHBX13	HW03007/05HWFHBX4+-++??	F2	-n++-?	06/07NZ

264	X05.620	06HWFHBX13	HW03007/05HWFHBX4+-++??	F2	-n---?	2007 bulk
265	X05.620	06HWFHBX13	HW03007/05HWFHBX4+-++??	F2	-n---?	2007 bulk
266	X05.621	06HWFHBX14	HW03007/05HWFHBX23++--??	F2	++nn-?	06/07NZ
267	X05.621	06HWFHBX14	HW03007/05HWFHBX23++--??	F2	++nn-?	06/07NZ
268	X05.622	06HWFHBX14	HW03007/05HWFHBX23++--??	F2	--nn+?	2007 bulk
269	X05.623	06HWFHBX14	HW03007/05HWFHBX23++--??	F2	--nn-?	2007 bulk
270	X05.623	06HWFHBX14	HW03007/05HWFHBX23++--??	F2	--nn-?	2007 bulk
271	X05.624	06HWFHBX15	05HWFHBX12/05HWFHBX1++--nn	F2	--nnnn	2007 bulk
272	X05.624	06HWFHBX15	05HWFHBX12/05HWFHBX1++--nn	F2	--nnnn	2007 bulk
273	X05.625	06HWFHBX15	05HWFHBX12/05HWFHBX1++--nn	F2	++nnnn	06/07NZ
274	X05.625	06HWFHBX15	05HWFHBX12/05HWFHBX1++--nn	F2	++nnnn	06/07NZ
275	X05.626	06HWFHBX16	05HWFHBX12/05HWFHBX20++--??	F2	++nn-?	06/07NZ
276	X05.627	06HWFHBX17	05HWFHBX12/05HWFHBX23++--??	F2	++nn-?	06/07NZ
277	X05.628	06HWFHBX17	05HWFHBX12/05HWFHBX23++--??	F2	--nn-?	2007 bulk
278	X05.629	06HWFHBX18	05HWFHBX13/05HWFHBX1++++nn	F2	----nn	2007 bulk
279	X05.629	06HWFHBX18	05HWFHBX13/05HWFHBX1++++nn	F2	----nn	2007 bulk
280	X05.630	06HWFHBX18	05HWFHBX13/05HWFHBX1++++nn	F2	--++nn	06/07NZ
281	X05.631	06HWFHBX18	05HWFHBX13/05HWFHBX1++++nn	F2	++--nn	06/07NZ
282	X05.632	06HWFHBX19	05HWFHBX13/05HWFHBX1++--nn	F2	--nnnn	2007 bulk
283	X05.633	06HWFHBX19	05HWFHBX13/05HWFHBX1++--nn	F2	++nnnn	06/07NZ
284	X05.634	06HWFHBX20	05HWFHBX16/05HWFHBX1++--nn	F2	++nnnn	06/07NZ
285	X05.635	06HWFHBX20	05HWFHBX16/05HWFHBX1++--nn	F2	--nnnn	2007 bulk
286	X05.635	06HWFHBX20	05HWFHBX16/05HWFHBX1++--nn	F2	--nnnn	2007 bulk
287	X05.636	06HWFHBX21	05HWFHBX16/05HWFHBX20++--??	F2	--nn+?	2007 bulk
288	X05.637	06HWFHBX21	05HWFHBX16/05HWFHBX20++--??	F2	++nn-?	06/07NZ
289	X05.638	06HWFHBX21	05HWFHBX16/05HWFHBX20++--??	F2	--nn-?	2007 bulk
290	X05.639	06HWFHBX22	05HWFHBX16/05HWFHBX23++--??	F2	++nn-?	06/07NZ
291	X05.640	06HWFHBX22	05HWFHBX16/05HWFHBX23++--??	F2	--nn-?	2007 bulk
292	X05.641	06HWFHBX22	05HWFHBX16/05HWFHBX23++--??	F2	--nn+?	2007 bulk
293	X05.642	06HWFHBX23	05HWFHBX18/05HWFHBX4+-++??	F2	-n---?	2007 bulk
294	X05.643	06HWFHBX23	05HWFHBX18/05HWFHBX4+-++??	F2	+n---?	2007 bulk
295	X05.644	06HWFHBX23	05HWFHBX18/05HWFHBX4+-++??	F2	+n++-?	AGGH SSD
296	X05.645	06HWFHBX24	05HWFHBX18/05HWFHBX20++--??	F2	++nn-?	06/07NZ
297	X05.646	06HWFHBX24	05HWFHBX18/05HWFHBX20++--??	F2	--nn-?	2007 bulk
298	X05.647	06HWFHBX25	05HWFHBX24/05HWFHBX1++--nn	F2	++nnnn	06/07NZ
299	X05.648	06HWFHBX25	05HWFHBX24/05HWFHBX1++--nn	F2	--nnnn	2007 bulk
300	X05.649	06HWFHBX26	05HWFHBX24/05HWFHBX4+-++??	F2	+n++-?	06/07NZ
301	X05.650	06HWFHBX26	05HWFHBX24/05HWFHBX4+-++??	F2	-n++-?	06/07NZ
302	X05.651	06HWFHBX26	05HWFHBX24/05HWFHBX4+-++??	F2	-n---?	2007 bulk
303	X05.652	06HWFHBX26	05HWFHBX24/05HWFHBX4+-++??	F2	+n---?	2007 bulk
304	X05.653	06HWFHBX27	05HWHBX3/05HWFHBX4	F2	+-++?	06/07NZ
305	X05.654	06HWFHBX28	05HWHBX3/05HWFHBX1	F2	++----	06/07NZ
306	X05.655	06HWFHBX29	05HWHBX3/05HWFHBX1	F2	++++--	AGGH SSD

Table 46. FHB marker evaluation of seven F5-derived hard white wheat populations

Cross ID	Pedigree	mark1	mark2	# lines tested	# lines retained
X05.575	BW315a*3/04GC0098	3BS	5A	345	28
X05.578	BW315a*3/Alsen	3BS	5A	157	28
X05.607	HW02061*3/04GC0177	3BS	5A	205	46
X05.615	HW02061*2//BW315a/Alsen	3BS	5A	170	3
X05.617	HW03007*3/Alsen	3BS	5A	163	17
X05.644	HW02061*2/04GC0176//BW315a*2/Alsen	3BS	5A	200	26
X05.655	BW315a*3/04GC0176//BW315a*2/04GC0098	3BS	5A	223	12
			total	**1463**	**160**

Table 47. Fusarium head blight (FHB) indices (2008) for lines selected for the 3BS (fhb1) and 5AS QTL

		FHB VRI			grain color		
Parents and populations	**Number of lines or replicates**	**Mean**	**SE**		**white**	**red**	**segregating**
BW315a	10	22	3.2	***			
X05.575	28	5	0.9		27	0	0
04GC0098	2	1	0.1	***			
Alsen	10	10	1.3	**			
BW315a		22		**			
X05.578	28	10	1.4		20	4	0
Alsen		10		NS			
HW0261	8	20	1.9	**			
X05.607	43	12	1.0		26	7	2
04GC0177	2	13	7	NS			
Alsen		10		NS			
HW03007	2	44	2.0				
X05.617	17	16	2.4	***	5	5	0
Alsen		10		*			
BW315a		22		P=0.07			
X05.644	26	15	2.3		11	5	0
04GC0176	2	28	4.5	NS			
Alsen		10		NS			
BW315a		22		***			
X05.655	12	7	1.3		0	0	11
04GC0176		28		NS			
04GC0098		1		***			
Alsen		10		NS			
X05.615	3	43	15				
HW0261		20		NS			
checks							
AC Cora	3	26					
AC Morse	3	46					
AC Vista	3	63					
CDC Teal	3	71					
FHB 37	3	5					
	LSD(0.05)	18					

Table 48. Fusarium head blight (FHB) indices (2009) for lines selected for the 3BS (fhb1) and 5AS QTL

		FHB VRI		
Parents and populations	**Number of lines or replicates**	**Mean**	**SE**	
BW315a	7	11.7	3.7	*
X05.575	27	1.3	0.3	
04GC0098	2	1.0	0.1	NS
Alsen	5	8.2	1.8	*
BW315a		11.7		P=0.11
X05.578	20	4.5	0.9	
Alsen		8.2		P=0.13
HW0261	4	11.3	2.6	P=0.18
X05.607	26	6.7	0.6	
Alsen		8.2		NS
HW03007	2	32.5	2.5	*
X05.617	5	4.8	0.6	
Alsen		8.2		P=0.15
BW315a		11.7		P=0.20
X05.644	11	6.1	1.0	
04GC0176	2	28.0	6.5	P=0.19
Alsen		8.2		NS
checks				
AC Cora	1	16		
AC Morse	1	38		
AC Vista	1	39		
CDC Teal	1	63		
FHB 37	1	6		

Figure 1. Percentage of grain germinated over time averaged over 4 reps, 2 runs, and 2 years for four different genotypes treated with four different seed treatments.

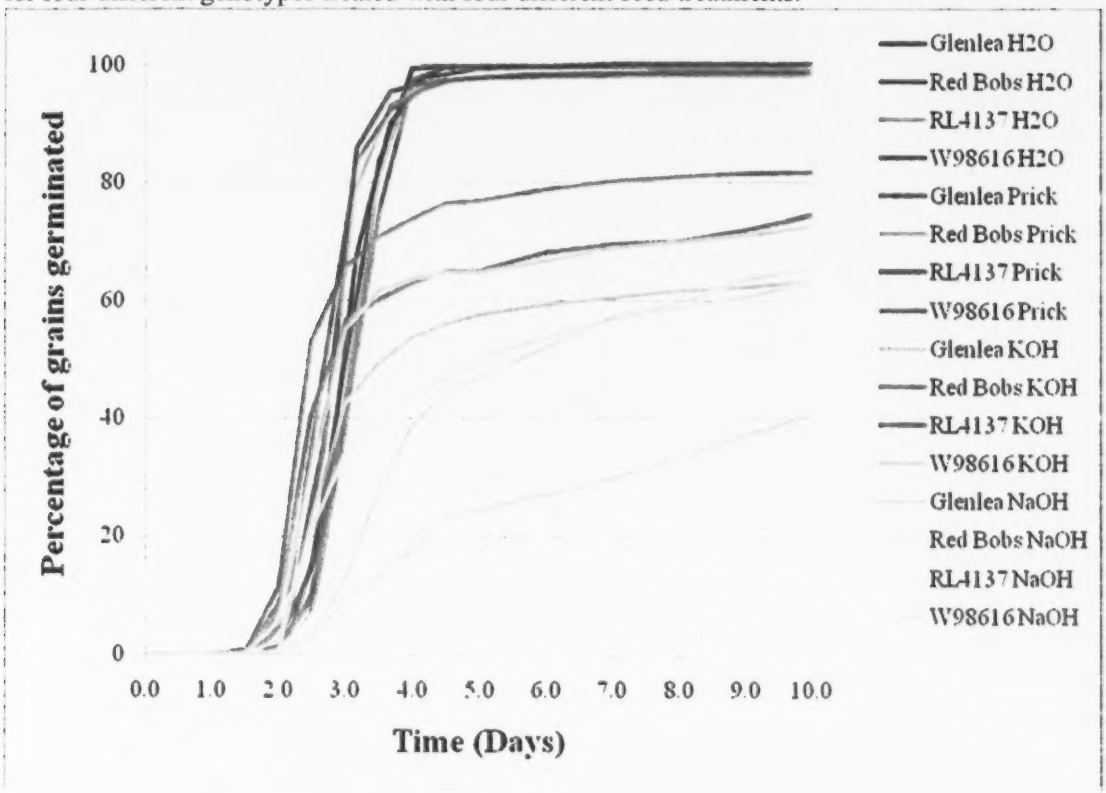
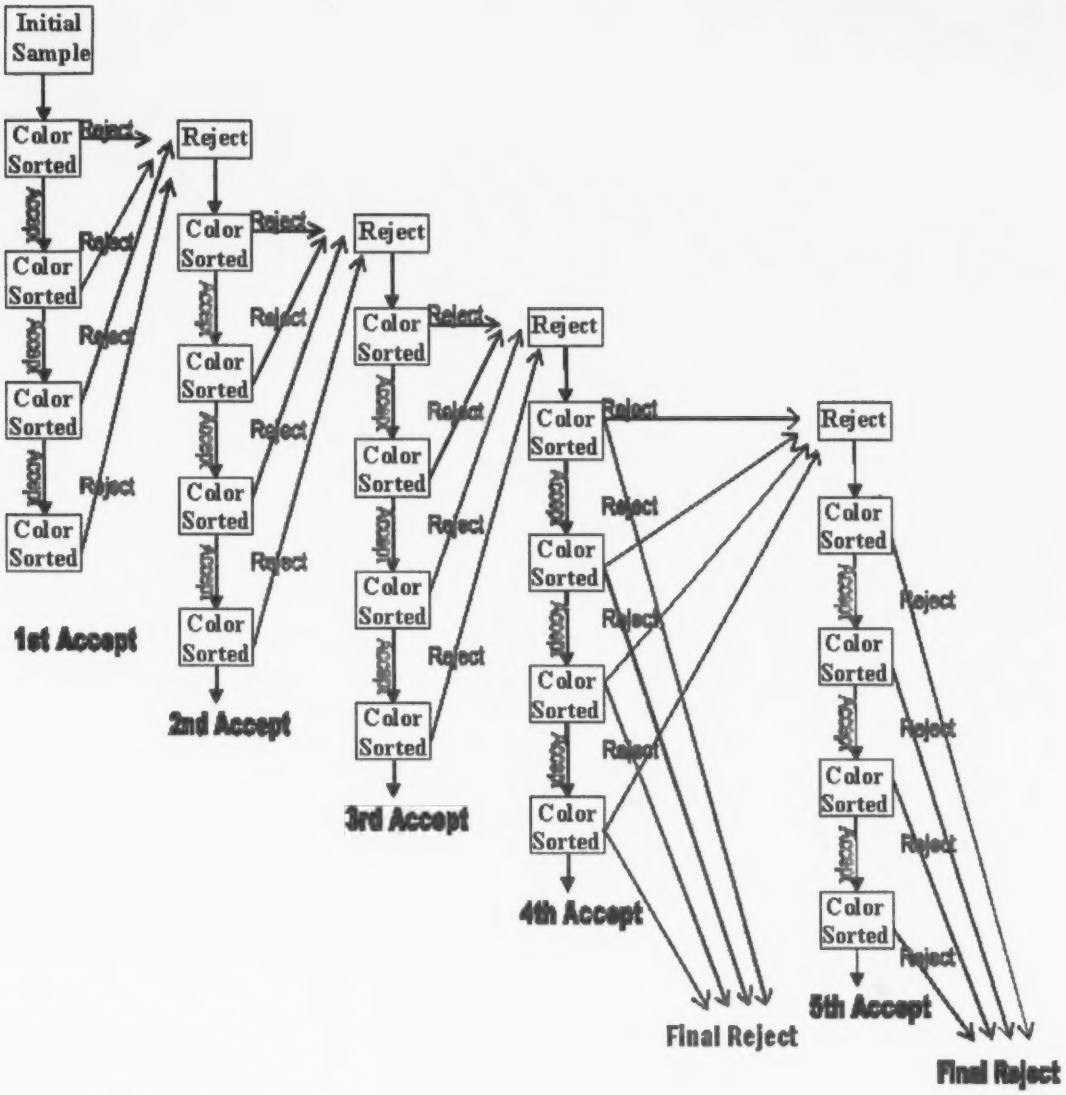
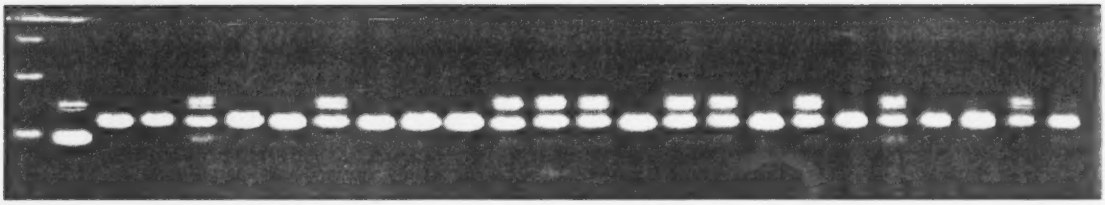


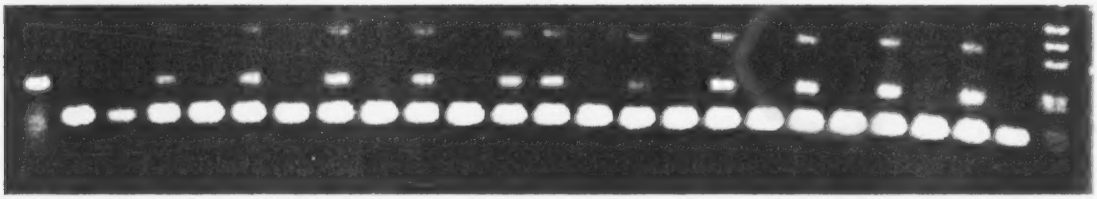
Figure 2. A schematic diagram displaying the process that a mixture of red and white wheat grain was color sorted by a Satake ScanMaster II color sorting machine to determine whether the marigold #1 background with the XZI filter lens or the cream #13 background with the XZI filter lens was superior in distinguishing red from white grain wheat (Final reject – blue) and if the machine could sort a 1 kg sample of wheat containing a ratio of 1 white grain: 63 red grain (Final reject – black).


Initial Sample
Color Sorted
Reject
Accept
1st Accept
2nd Accept
3rd Accept
4th Accept
5th Accept
Final Reject
Final Reject

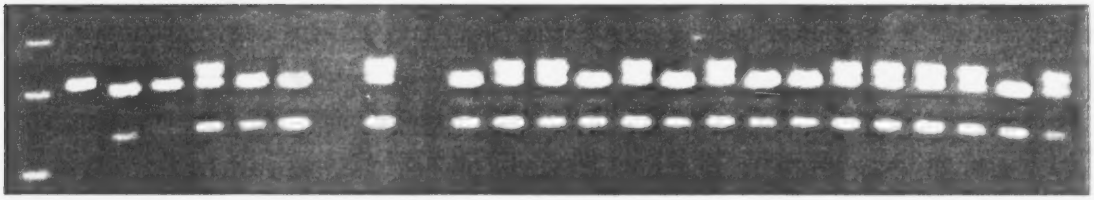
A) gwm533



B) gwm493



C) gwm304



D) gwm293

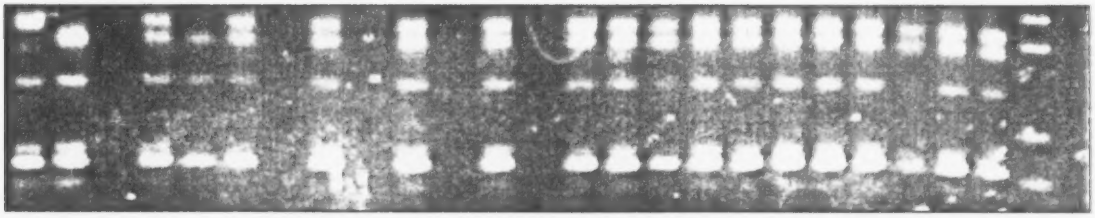


Fig. 3: Amplification products, using four microsatellite primers observed in FHB resistant (Sumai-3) and susceptible (BW315a) genotypes and separated on ethidium stained agarose gels. For Sumai-3 and BW315a, respectively, amplicon sizes were 160bp and 133bp for locus *Xgwm533-3BS;* 213bp and 157bp for *Xgwm493-3BS;* 233bp and 217bp for *Xgwm304-5AS;* and 218bp and 208bp for *Xgwm293-5AS.* In (A) lanes 1=DNA ladder; 2=Sumai-3; 3=BW315a; and 5, 8, 12-14, 16, 17, 19, 21, 24= heterozygous at locus *Xgwm533-3BS.* In (B) lanes 1=Sumai-3; 2=BW315a; and 4, 6, 8, 10, 12, 13, 15, 17, 19, 21, 23= heterozygous at *Xgwm493-3BS.* In (C) lanes 1= DNA ladder; 2=Sumai-3; 3=BW315a; and 5, 9, 12, 13, 15, 17, 20-23, 25= heterozygous at *Xgwm304-5AS.* In (D) lanes 1=Sumai-3; 2=BW315a; and 4, 6, 8, 10, 12, 14-24= heterozygous at *Xgwm293-5AS.* In (A) and (C), the first lanes illustrate the 100, 200, and 300 bp fragments observed for the 1 Kb Plus DNA ladder. In (B), the last lane illustrates the 220, 298, 344, and 394 bp fragments corresponding to the 1 Kb DNA ladder. In (D), the last lane illustrates the 134, 154, 201, and 220 bp fragments corresponding to the 1 Kb DNA ladder.